Proc. Nat. Acad. Sci. USA
Vol. 69, No. 10, pp. 2986–2990, October 1972

A Transcribing Activity Induced by Satellite Phage P4

(helper-dependent/RNA polymerase/DNA synthesis)

KATHLEEN J. BARRETT, WARREN GIBBS, AND RICHARD CALENDAR

Department of Molecular Biology, University of California, Berkeley, Calif. 94720

Communicated by Howard K. Schachman, August 7, 1972

ABSTRACT **Satellite bacteriophage P4 induces a new transcribing enzyme that synthesizes polyriboguanylic acid in the presence of the poly(dG)·poly(dC) homopolymer pair. This transcribing activity was partially purified and shown to be distinct from the host RNA polymerase. Analysis of conditional lethal mutants suggests that this new enzyme is necessary for replication of phage DNA.**

Satellite bacteriophage P4 requires a helper phage, such as temperate phage P2, for lytic multiplication (1). The P2 helper genome may be present either as a coinfecting phage or as a prophage (2). If it is coinfecting, both P2 and P4 are produced in the resultant burst; while if it is a prophage, only P4 is produced (2). P4 requires all 18 of the P2 helper late genes (ref. 3) and the completed phage contains P2 head and tail proteins (4).

In a normal P2 infection, DNA synthesis and early gene expression are required for activation of P2 late genes (5–9). In contrast, P4 requires neither P2 DNA synthesis nor P2 early functions to activate P2 late genes (3, 10). Furthermore, P4 can induce late gene products from a repressed P2 prophage (2), whereas helper phage P2 cannot induce significant amounts of late gene products from a heteroimmune prophage (11). Thus, the mechanism by which the two phage induce P2 late genes appears to differ. We considered the possibility that P4 might induce a new RNA polymerase with the specificity to transcribe late genes of the helper phage. Such a mechanism is used by phage T7 to activate transcription of its late genes (12). While searching for a new RNA polymerase activity in P4-infected cells, we found a P4-induced transcribing activity that synthesizes poly(G) with poly(dG)·poly(dC) as template. A study of P4 conditional lethal mutants (13) suggests that this enzyme is not involved in transcription of the P2 helper genome. Instead, it appears to be essential for P4 DNA replication. We report here some properties of the partially purified enzyme.

MATERIALS AND METHODS

Radioactively labeled nucleosides and nucleotides were purchased from Schwartz/Mann, and unlabeled nucleotides from Calbiochem. Rifamycin (rifampin) was donated by CIBA Pharmaceutical Co. or was from Calibiochem. Streptolydigin was donated by the Upjohn Corp. Mitomycin C was obtained from Sigma Chemical Co., and actinomycin D, from Mann Research Laboratories. Poly(dA–dT) and T7 DNA were gifts from Michael Chamberlin. Poly(dG–dC) was a gift from R. D. Wells. Poly(dG)·poly(dC) was prepared according to Radding *et al.* (14), with *Escherichia coli* DNA polymerase I kindly provided by A. Kornberg. Serum containing antibodies to DNA polymerase was a gift from D. Brutlag. P2 and P4 phage preparations, which had been banded twice in CsCl density gradients, were used for isolation of DNA (15). The integrity of phage DNA was measured by alkaline zonal sedimentation in an analytical ultracentrifuge (16).

Medium. Medium A (17) and TPG–CAA medium (18) were used.

Bacteriophage Strains. P2 tsK_{60} old_1 carries a temperature-sensitive mutation in the lysis gene K and cannot interfere with growth of phage lambda (19); P2 vir_1 am_{12} is a virulent strain that carries an amber mutation in a lysis gene (20); P2 cox_1 is an excision-defective mutant (21); P4 vir_1 is a clear plaque-type mutant (18); P4 vir_1 $amA_{1,25\text{ and }26}$ and P4 vir_1 tsB_{57} are described by Gibbs *et al.* (13).

Bacterial Strains. All strains were derivatives of *E. coli* C (22). C-1a and C-2 are prototrophic (23, 24); C-10 lacks endonuclease I (25); C-520 carries amber suppressor D (*supD*) (26); C-1748 is C-1a, which was lysogenized with P2 cox_1; C-1847 is C-10, which was lysogenized with P2 tsK_{60} old_1; HF4704 is thymine-requiring and cannot reactivate ultraviolet light damage to DNA(hcr^-) (27); C-1971 is a rifamycin-permeable mutant of HF4704 (28); C-1859 is an F^+ strain derived from C-1971 by contact with the F^+ K12 strain, CR34, which was obtained from D. Helinski.

Growth of Infected Cells in the Biogen (American Sterilizer Co.). 40 Liters of C-1847 were grown in A medium at 33° in the biogen to a cell density of 1×10^9 cells per ml. The cells were infected with P4 vir_1 at a multiplicity of infection (MOI) of 0.15, and growth at the permissive temperature was continued for 70 min at 33° to allow expression of the lysis function in the first cycle of growth. The temperature was then raised to 42° to prevent lysis during the second round of P4 multiplication. After 100 min at the nonpermissive temperature, the infected cells were forced from the biogen into a vessel containing ice. The chilled cells were harvested by centrifugation in a Beckman model 170 continuous flow centrifuge and were stored at −20°.

Preparation of Cell Extracts. All operations were performed at 0–4°. Cells were disrupted either by alumina grinding or by sonication. In the alumina grinding procedure, 0.3–1.5 g of cells were ground for 10 min on ice with twice the weight of washed alumina followed by extraction with 1 ml of buffer A per g of cells. Buffer A contains 50 mM Tris (pH 8.0),10 mM $MgCl_2$, 0.1 mM dithiothreitol, 0.1 mM EDTA, 5% glycerol. In the sonication procedure, 0.1–1.5 g of frozen cells were homogenized with about 2 ml of buffer A per g of cells, and the suspension was sonicated with a Biosonic II for 1.5 min

Abbreviation: MOI: multiplicity of infection.

TABLE 1. *Drug-resistant RNA polymerase activity in phage-infected cells*

Host	Phage	Specific activity (pmol incorporated/mg per min)	% Activity resistant to rifamycin and streptolydigin
Nonlysogenic (C-10)	None	1000	2.6
	P4 vir_1	1000	16
Nonlysogenic (C-1a)	None	1000	2.5
	P2 vir_1 am_{12}	1000	2.5
P2-lysogenic (C-1748)	None	500	5.8
	P4 vir_1	1220	31

Cells were grown to a density of 5×10^8/ml and were infected at an MOI of 10 in TPG–CAA medium (C-10) or A medium (C-1a and C-1748). Infected cells were incubated with aeration at 37° until the late stages of the infectious cycle were reached, i.e., 25 min for P2-infected C-1a, 50 min for P4-infected C-1748, and 100 min for P4-infected C-10. Cells were harvested by centrifugation and frozen. Extracts were prepared by alumina grinding (C-1a and C-10) or sonication (C-1748) and were assayed for total activity and drug-resistant activity.

at 0°. Cellular debris was removed by centrifugation for 30 min at 15,000 × *g*. Protein concentrations were determined according to Lowry *et. al.* (29).

Assays of Transcribing Activity were performed essentially as described by Chamberlin *et al.* (12). The 0.1-ml "standard" assay mixture contained 40 mM Tris pH 8.0, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 1 mg/ml bovine serum albumin, 0.1–0.2 mM poly(dG)·poly(dC), and 0.2 mM [^{3}H]GTP (specific activity 30–70 cpm/pmol). "Drug-resistant activity" was measured by addition of 20 μg/ml rifamycin and 0.2 mM streptolydigin to the standard assay. All reactions were terminated after 10 min at 37° by addition of 3 ml of cold 3.5% perchloric acid containing 0.1 M sodium pyrophosphate. Acid-insoluble material was collected on a Whatman GF/C filter, washed extensively with cold 1 M HCl containing 0.1 M sodium pyrophosphate and then with 95% ethanol. The filters were dried and counted in a toluene-based scintillation fluid.

RESULTS

Evidence for a New Transcribing Activity. A new transcribing activity can be detected in P4-infected cells when the host RNA polymerase is inactivated by rifamycin and streptolydigin (Table 1). This activity is assayed as incorporation of [^{3}H]GTP into an acid-insoluble form in the presence of poly(dG)·poly(dC). In extracts prepared from uninfected cells, less than 5% of the RNA polymerase activity is drug-resistant. However, when the extracts are prepared from P4-infected cells, 15–40% of this activity is resistant to the drugs. Thus, P4-infected cells contain an RNA polymerase activity that can be distinguished from the host activity by its drug resistance.

Several lines of evidence suggest that this enzyme activity is induced by P4. First, this activity is found in P4-infected cells whether or not the P2 helper phage is present. It is not found in cells that are infected with P2 alone (Table 1). Secondly, this activity is not found in cells infected with certain P4 mutants. Under nonpermissive conditions, P4 amber mutants in cistron A (13) do not synthesize detectable amounts of this activity (Table 2). However, under permissive conditions, a significant amount of the poly(dG)·poly(dC)-dependent GTP incorporation was drug resistant. We therefore conclude that this new activity is induced by the P4 genome.

TABLE 2. *RNA polymerase activity in Sup$^+$ and Sup$^-$ cells infected with P4 amber mutants*

Host	Phage	Specific activity (pmol incorporated/mg per min)	% Activity resistant to rifamycin and streptolydigin
Sup$^-$(C-2)	None	183	3.9
	P4 vir_1 amA_1	63	4.9
	P4 vir_1 amA_{25}	140	3.7
	P4 vir_1 amA_{26}	210	3.1
Sup$^+$(C-520)	None	174	1.6
	P4 vir_1 amA_1	364	15
	P4 vir_1 amA_{25}	76	43
	P4 vir_1 amA_{26}	64	53

Cells were grown to a density of 2×10^8/ml in A medium and were infected at an MOI of 10. The cells were harvested by centrifugation 100 min later and were treated as described in Table 1.

Properties of the P4 Transcribing Activity. Sedimentation of partially purified P4 enzyme on glycerol gradients shows that the new activity is physically, as well as biochemically, distinct from *E. coli* RNA polymerase (Fig. 1*a*). The poly(G)-synthesizing activity sediments in two peaks. One of these has a sedimentation coefficient of about 14 S and is similar to the RNA polymerase from uninfected cells in its drug sensitivity (5–10% resistant) and in its activity with several DNA templates. The slower sedimenting material is the P4-induced activity. It is at least 90% drug-resistant (see also Table 3) and is not found in uninfected cells (Fig. 1*b*). It has a sedimentation coefficient of 5 S, as determined by comparison with alkaline phosphatase (data not shown) and with *E. coli* RNA polymerase (Fig. 1*b*), assuming an $s_{20,w}$ for these standards of 6.1 S (30) and 13.5 S (31), respectively.

The reaction was studied with partially purified 5S enzyme obtained from glycerol gradients (Table 3). The enzyme is again seen to differ from *E. coli* RNA polymerase in its resistance to rifamycin and streptolydigin. It is antigenically distinct as well, since it is resistant to antibody directed against *E. coli* RNA polymerase. The incorporation of GTP is dependent on the addition of poly(dG)·poly(dC) and is sensitive to actinomycin D. The requirement for a divalent cation is satisfied by Mg^{++}, but only partially by Mn^{++} (data not shown). The product of the reaction is more than 95% sensitive to treatment with alkali as would be expected for a ribopolymer. These results suggest that the P4 transcribing activity is a new enzyme that catalyzes the poly(dG)·poly(dC)-dependent incorporation of GTP into poly(G).

Biological Function of the New Activity. If the enzyme is involved in transcription of phage DNA, then natural DNA

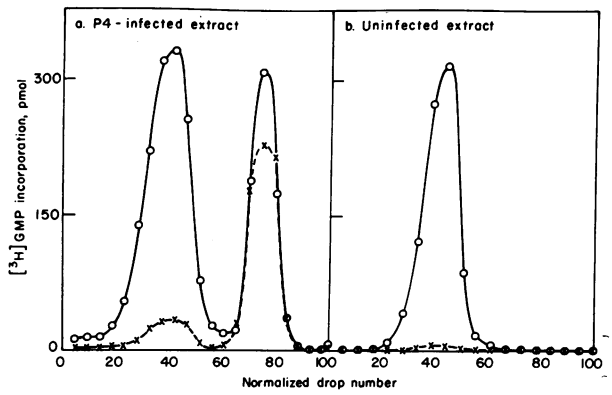

FIG. 1. Sedimentation of the poly(dG)·poly(dC) transcribing activity from P4-infected and uninfected cells. Sedimentation is from *right* to *left*. Bacteria (C-1847) were grown in the Biogen. About 1 g of P4-infected or uninfected cells was sonicated with 5 ml of buffer A at 0°. Cellular debris was removed by centrifugation for 10 min at 15,000 × *g*, and the pellet was washed with 1 ml of buffer A. The combined supernatant fractions were centrifuged for 30 min at 17,000 × *g*. The supernatant fraction was centrifuged for 100 min at 105,000 × *g* and the supernatant was discarded. RNA polymerase activity was eluted from the pellet by incubation for 60 min on ice with 0.6 ml of buffer A supplemented with KCl to 2M. The sample was again centrifuged at 105,000 × *g* for 100 min. The supernatant fraction was concentrated by precipitation with 1.5 volumes of saturated (25°), neutralized ammonium sulfate. The ammonium sulfate pellet was collected after centrifugation for 20 min at 15,000 × *g* and dissolved in 0.25 ml of 10 mM Tris(pH 8.0), 10 mM $MgCl_2$, 0.1 mM dithiothreitol, 0.1 mM EDTA. This fraction (0.2 ml) was layered onto a 10–30% glycerol gradient prepared in the same buffer supplemented with 0.2 M KCl and 1 mg/ml bovine-serum albumin and was centrifuged for 11.5 hr at 40,000 rpm in an SW-50 rotor in a Spinco model L ultracentrifuge. The centrifuge tube was punctured and 4-drop fractions were collected. 10 μl of each fraction was assayed for activity in the presence (×—×) and the absence (O—O) of rifamycin and streptolydigin. Assays were conducted under standard conditions except that the volume of the assay was reduced to 0.05 ml and 1 mM phosphate was added.

templates ought to be able to substitute for the synthetic polymer, poly(dG)·(dC). We therefore investigated the template specificity of the two species of RNA polymerase from P4-infected cells (Table 4). The 14S enzyme from P4-infected cells resembles *E. coli* RNA polymerase in its template specificity. However, the new 5S activity shows a pronounced preference for poly(dG)·poly(dC) as template. Its activity with P2, P4, and T7 DNA is less than or equal to 1% of its activity with poly(dG)·poly(dC). No detectable activity is obtained with poly(dA–dT) or poly(dG–dC) templates (Table 3). Furthermore, poly(dG)·poly(dC) promotes the synthesis of poly(G) and not the synthesis of poly(C). Thus, the enzyme is highly specific.

This high degree of specificity and the inability of the new enzyme to transcribe native biological DNA is open to several interpretations. In order to distinguish between some of these possibilities, we sought more information on the function of the enzyme by studying whether or not the enzyme is synthesized by the two known classes of conditional lethal P4 mutants (13). Mutants in class B are represented by a single temperature-sensitive mutant, P4 vir_1 tsB_{37}. The P4 B mutant

TABLE 3. *Requirements of the new transcribing activity*

Experiment	Conditions	pmol of GMP incorporated
1.	Complete	125
	− poly(dG)·poly(dC)	1
	+ rifamycin and streptolydigin	131
	− enzyme	1
	Alkali digested	3
2.	Complete	151
	− enzyme	3
	+ actinomycin D	2
	+ antibody to *E. coli* RNA polymerase	143

The complete reaction mixture contained 1 mM phosphate in addition to the components of the standard assay. Drugs were added as indicated. Actinomycin D was present at 40 μg/ml. This high concentration of actinomycin was also necessary to completely inhibit *E. coli* RNA polymerase with poly(dG)·poly(dC) template in agreement with the results of Wells and Larson (39). The amount of antibody present in the reaction (0.04 A_{280} units) decreased the incorporation obtained with *E. coli* RNA polymerase from 202 to 36 pmol. The product of the reaction was digested with alkali as follows: The reaction was terminated by addition of 0.1 ml of 1 N NaOH and the mixture was digested for 2 hr at 37°. The sample was chilled and neutralized with 0.1 ml of 1 N HCl. Acid-insoluble radioactive material was determined as described for the standard assay. Enzyme (10 μl) was from the 5S peak of gradients prepared as described in Fig. 1*a*.

synthesizes P4 DNA under nonpermissive conditions, and it can activate transcription of the P2 genome (13). This temperature-sensitive mutant synthesized small but readily detectable amounts of enzyme at the nonpermissive temperature (Fig. 2*a*). The lower level of activity observed with P4 vir_1 tsB_{37} is probably due to a general lowering of macromolecular synthesis in cells infected with this mutant (13). Mutants in class A do not synthesize the new enzyme (Table 2; Fig. 2*b*) nor do they synthesize P4 DNA. However, these mutants do activate P2 late genes since late mRNA as well as phage heads are found under nonpermissive conditions (13).* These experiments suggest that the new enzyme is involved in synthesis of P4 DNA and not in transcription of helper phage genes.

The above genetic data forced us to ask whether the P4-induced enzyme might be a DNA polymerase. We found that the 5S enzyme fraction can incorporate [³H]dGTP (Table 5). However, all of this poly(dG) synthesizing activity is eliminated by antiserum to DNA polymerase I. In contrast, the synthesis of poly(G) is not affected by antiserum (Table 5, Exp. 2). Contamination of the 5S fraction with *E. coli* DNA polymerase I can therefore account for the synthesis of poly(dG), but not for the synthesis of poly(G). Thus, the phage-induced enzyme appears to be an RNA polymerase that is required for the synthesis of P4 DNA.

Recent results from several laboratories (32–37) suggest a role for transcriptional activity in synthesis of DNA. For

* The effect of P4 A gene amber mutants on enzyme synthesis is probably not due to a polar effect, since similar results are obtained in a host strain carrying a polarity suppressor (26).

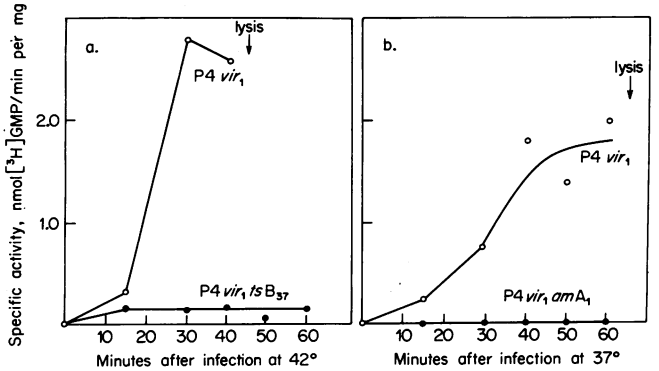

FIG. 2. Kinetics of appearance of the P4-induced transcribing enzyme. *E. coli* C-1748 was grown in A medium to a density of 5×10^8 cells per ml. The cells were concentrated 10-fold in A medium that lacked glucose and amino acids and infected with P4 vir_1, P4 vir_1 amA_1, or P4 vir_1 tsB_{37} at an MOI of 10. The phage were allowed to adsorb on ice for 20 min. The cells were then diluted 20-fold into warmed A medium and were aerated at 37° or 42°. Samples (75 ml) were taken at the times indicated and were rapidly chilled. The cells were pelleted by centrifugation and stored frozen at −15°. Cell extracts were prepared by sonication and were assayed for drug-resistant activity. Lysis of the cultures was monitored with a Klett spectrophotometer (600 nm).

example, Brutlag *et al.* (33) find that replication of M13 DNA is more sensitive to rifamycin than to chloramphenicol, and suggest that initiation of DNA replication might involve transcription of a primer RNA by the host RNA polymerase. If the new P4 transcribing activity were required for P4 DNA synthesis, then P4, unlike M13, should be rifamycin-insensitive in the replication of its DNA. That this is so is shown in Fig. 3. DNA replication in P4 is much less sensitive to rifamycin than to chloramphenicol under conditions in which the replication of fd DNA [fd is very closely related to M13 (38)] is much more sensitive to rifamycin than to chloramphenicol. This finding is consistant with the interpretation that the new rifamycin-resistant activity might provide a primer transcript that is required for replication of P4 DNA.

DISCUSSION

We have found a new transcribing activity in *E. coli* infected with satellite phage P4. This enzyme appears to be specified by the P4 genome, since it is produced in the presence and in the absence of the helper phage P2 (Table 1) and is not produced by P4 phage that carry a mutation in gene A (Table 2). This new transcribing activity appears to be unrelated to *E. coli* RNA polymerase, since it is resistant to antibody directed against the *E. coli* enzyme and to rifamycin and streptolydigin (Table 3), and sediments at a slower rate (Fig. 1). Furthermore, this P4-induced enzyme is highly specific in that it transcribes only poly(G) from poly(dG)·poly(dC) template.

P4 mutants that do not synthesize this enzyme are also unable to replicate P4 DNA, although they are able to activate transcription of the required P2 helper genes (13). Thus, the new transcribing activity may be the product of P4 gene A and may be required for synthesis of P4 DNA. However, we cannot exclude the possibility that P4 gene A regulates synthesis of this enzyme, and that the enzyme is nonessential.

TABLE 4. *Template specificity of RNA polymerase*

	pmol GMP incorporated		
	Infected		Uninfected
Template	5 S	14 S	14 S
Experiment 1			
None	1.5	5.5	3.8
Poly(dG)·poly(dC)	152	253	270
P2 DNA	2.3	39	68
P4 DNA	1.9	58	96
T7 DNA	2.3	66	154
Experiment 2			
None (ATP)	2.5	2.6	2.6
None (GTP)	0.7	3.7	2.9
poly(dG)·poly(dC)	134	255	335
poly(dA–dT)	2.6	621	633
Experiment 3			
None (*GTP + CTP)	1.0	1.0	
None (GTP + *CTP)	2.0	2.9	
Poly(dG)·poly(dC) (*GTP)	140	218	
Poly(dG)·poly(dC) (*GTP + CTP)	106	136	
Poly(dG)·poly(dC) (GTP + *CTP)	3.0	66	
Poly(dG–dC) (*GTP + CTP)	1.0	39	

All assay mixtures contained 1 mM phosphate. Assays with poly(dG)·poly(dC) were performed under standard conditions with only GTP present unless otherwise indicated. Assays with phage DNA as template (Exp. 1) contained ATP, UTP, CTP (0.4 mM each), [³H]GTP (0.2 mM, 65 cpm/pmol), and the appropriate phage DNA (0.3 mM). Activity with poly(dA–dT) (0.15 mM) as template (Exp. 2) was measured with 0.4 mM UTP and 0.4 mM [¹⁴C]ATP (10 cpm/pmol). Reactions without template contained [¹⁴C]ATP or [³H]GTP as indicated in parentheses. In Exp. 3, the nucleotides (0.2 mM) present in the reaction mixture were varied as indicated in parentheses. The radioactive nucleotide (55 cpm/pmol) is designated with an *asterisk*. Poly(dG–dC) was present at 0.05 mM and poly(dG)·poly(dC) at 0.15 mM. Enzyme (10 μl) was obtained from the appropriate regions of gradients prepared as described in Fig. 1.

TABLE 5. *Sensitivity of the P4 enzyme to DNA polymerase antiserum*

Experiment	Substrate	Conditions	pmol incorporated
1.	[³H]dGTP	Complete	39.0
		+ 20 μl DNA polymerase I antiserum	2.2
2.	[³H]GTP	Complete	14.8
		+ 20 μl DNA polymerase I antiserum	15.6

The reaction mixture used for measurement of [³H]dGTP incorporation was that described by Radding *et al.* (14) for the preparation of poly(dG)·poly(dC) except that the concentration of triphosphates was lowered to 30 μM and 2-mercaptoethanol was present at 1 mM. Under these conditions, 10 μl of DNA polymerase antiserum was sufficient to reduce the incorporation obtained with purified DNA polymerase I from 190 pmol to 27 pmol. Standard assay conditions were used in Exp. 2. Enzyme (10 μl) was from the 5S region of a gradient prepared as described in Fig. 1*a*.

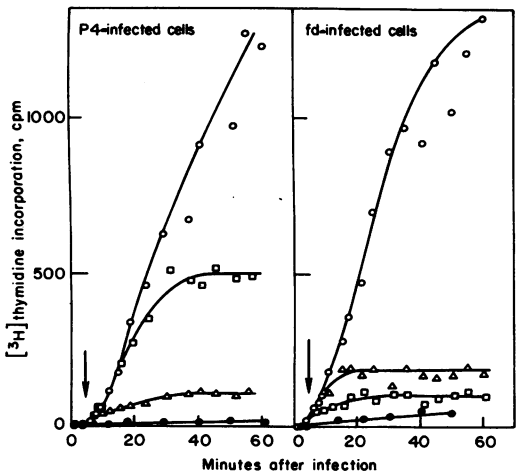

FIG. 3. Sensitivity of DNA synthesis in P4- and fd-infected cells to rifamycin and chloramphenicol. *E. coli* C-1859 was grown to a concentration of 1.5 to 2 × 10^8/ml in TPG–CAA medium supplemented with 10 μg/ml of thymidine. Host DNA synthesis was suppressed by treatment of the cells with mitomycin C by a modification of the procedure described by Lindqvist and Six (18). Mitomycin C was removed by Millipore filtration. The cells were washed with and concentrated 10 times in TPG-CAA medium lacking glucose and casamino acids, but containing 2.5 μg/ml thymidine. Portions of the cells were infected with P4 or fd (MOI of 10) and were incubated for 5 min at 37° without aeration to allow adsorption of the phage. The cells were then diluted 10-fold into warmed TPG–CAA medium containing 2.5 μg/ml of thymidine. Uninfected cells (5 ml) and infected cells (15 ml) were added to [^{3}H]thymidine (5 μCi/ml, 18.5 Ci/mmol). Both samples were aerated at 37° (time = zero). 5 min later, 5-ml aliquots of infected cells were removed to flasks containing chloramphenicol or rifamycin (final concentration of 100 μg/ml and 60 μg/ml, respectively). 50-μl Samples were removed at the times indicated to 2-cm circles of Whatman 3 MM filter paper and dropped into 5% trichloroacetic acid. The filter papers were batch-washed and counted essentially as described by Bollum (40). Uninfected cells, ●—●; phage-infected cells, ○—○; infected cells to which chloramphenicol (△—△) or rifamycin (□--□) were added 5 min after infection.

There is mounting evidence that a transcriptional event is required for DNA synthesis in coliphages λ (32) and M13 (33, 34), in colicin E_1 (35), and in F-factor (36) as well as in *E. coli* (37). On the basis of our data we suggest that the highly specific P4 transcribing enzyme recognizes the replication origin of P4 DNA and synthesizes a polyribonucleotide that serves as the primer for DNA synthesis.

We thank Michael Chamberlin for the use of his laboratory facilities for our initial experiments and for suggesting the use of poly(dG)·poly(dC). We appreciate the skilled assistance of Roberta Wiener and Ted Burch. This work was supported by U.S. Public Health Service Research Grants AI 08722 and AI 01267 from the National Institute of Allergy and Infectious Diseases, by Training Grant GM 01389 from the National Institute of General Medical Sciences, by Training Grant CA 05028 from the National Cancer Institute, by Cancer Contract 71-2173 from the National Cancer Institute Special Virus-Cancer Program, by Grant 507 from the California Division of the American Cancer Society, and by Grant 217 from the Jane Coffin Childs Memorial Fund for Medical Research.

1. Six, E. W. (1963) *Bacteriol. Proc.* 138.
2. Six, E. W. & Klug, C. A. C. (1972) *Virology*, in press.
3. Six, E. W. & Lindqvist, B. H. (1970) *Bacteriol. Proc.* 202.
4. Gibbs, W. (1972) Thesis, University of California, Berkeley.
5. Lindahl, G. (1970) *Virology* **42**, 522–533.
6. Lindqvist, B. H. (1971) *Mol. Gen. Genet.* **110**, 178–196.
7. Lindahl, G. (1972) *Mol. Gen. Genet.*, in press.
8. Lindqvist, B. H. & Bøvre, K. (1972) *Virology*, in press.
9. Geisselsoder, J. (1972) Ph.D. Thesis, University of Hawaii, Honolulu.
10. Six, E. W. & Lindqvist, B. H. (1971) *Virology* **43**, 8–15.
11. Six, E. W. (1971) *Bacteriol. Proc.* 180.
12. Chamberlin, M. J., McGrath, J. & Waskell, L. (1970) *Nature* **228**, 227–231.
13. Gibbs, W., Goldstein, R. N., Wiener, R., Lindqvist, B. H. & Calendar, R. L. (1972) *Virology*, in press.
14. Radding, C. M., Josse, J. & Kornberg, A. (1962) *J. Biol. Chem.* **237**, 2869–2876.
15. Thomas, C. A. & Abelson, J. (1966) in *Procedures in Nucleic Acid Research*, eds. Cantoni, G. L. & Davies, D. R. (Harper & Row, New York), pp. 553–561.
16. Studier, F. W. (1965) *J. Mol. Biol.* **11**, 373–390.
17. Bertani, L. E. & Bertani, G. (1970) *J. Gen. Virol.* **6**, 201–212.
18. Lindqvist, B. H. & Six, E. W. (1971) *Virology* **43**, 1–7.
19. Lindahl, G. (1969) *Virology* **39**, 839–860.
20. Lindahl, G. (1971) *Virology* **46**, 620–633.
21. Lindahl, G. & Sunshine, M. (1972) *Virology* **49**, 180–187.
22. Bertani, G. & Weigle, J. J. (1953) *J. Bacteriol.* **65**,113–121.
23. Sasaki, I. & Bertani, G. (1965) *J. Gen. Microbiol.* **40**, 365–376.
24. Bertani, G. & Six, E. W. (1958) *Virology* **6**, 357–381.
25. Dürwald, H. & Hoffman-Berling, H. (1968) *J. Mol. Biol.* **34**, 331–346.
26. Sunshine, M. G., Thorn, M., Gibbs, W., Calendar, R. & Kelly, B. (1971) *Virology* **46**, 691–702.
27. Lindqvist, B. H. & Sinsheimer, R. L (1967) *J. Mol. Biol.* **30**, 69–80.
28. Ljungkvist, E. (1972) *Virology*, in press.
29. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
30. Schlesinger, M. & Barrett, K. (1965) *J. Biol. Chem.* **240**, 4284–4292.
31. Berg, D. & Chamberlin, M. J. (1970) *Biochemistry* **9**, 5055–5064.
32. Dove, W. F., Inokuchi, H. & Stevens, W. F. (1971) in *The Bacteriophage Lambda*, ed. Hershey, A. D. (Cold Spring Harbor Laboratory, New York), pp. 747–771.
33. Brutlag, D., Schekman, R. & Kornberg, A. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2826–2829.
34. Wickner, W., Brutlag, D., Schekman, R. & Kornberg, A. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 965–969.
35. Clewell, D. B., Evenchik, B. & Cranston, J. W. (1972) *Nature New Biol.* **237**, 29–30.
36. Bazzicalupo, P. & Tocchini-Valentini, G. P. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 298–300.
37. Lark, K. G. (1972) *J. Mol. Biol.* **64**, 47–60.
38. Marvin, D. & Hohn, B. (1969) *Bacteriol. Rev.* **33**, 172–209.
39. Wells, R. D. & Larson, J. E. (1970) *J. Mol. Biol.* **49**, 319–342.
40. Bollum, F. J. (1966) in *Procedures in Nucleic Acid Research*, eds. Cantoni, G. L. & Davies, D. R. (Harper & Row, New York), pp. 296–300.