INDUCTION OF A NEW RNA POLYMERASE IN ESCHERICHIA COLI INFECTED WITH BACTERIOPHAGE T3

Umadas Maitra

Department of Developmental Biology and Cancer
Division of Biological Sciences
Albert Einstein College of Medicine
Bronx, New York 10461

Received March 19, 1971

SUMMARY: A new T3-specific RNA polymerase is found in T3-phage infected E. coli cells. Evidence is presented to show that the phage-induced polymerase is physically and biochemically distinct from the host cell RNA polymerase. The phage polymerase utilizes T3 DNA as template and is ineffective with T4, T7, calf thymus DNA or dAT copolymer. The polymerase can however copy dG: dC homopolymer, making poly rG exclusively.

INTRODUCTION: It is now well established that the expression of genetic information in bacteriophages, as well as in more complex organisms, is subject to temporal control; not all genes are expressed simultaneously. Early and late functioning cistrons have been distinguished in λ, T-even and other bacteriophages. In these viruses, temporal regulation of gene expression seems to occur at the transcriptional level. At a certain time after infection, mRNA synthesis, which originally was specific for early phage proteins, is altered to produce RNA which directs the synthesis of late phage proteins. Evidence for this control mechanism has been reviewed (1,2) . In the case of phage T4, it has been shown (2-7) that the change in transcription specificity leading to the synthesis of late mRNA is achieved by the synthesis of a new sigma subunit of E. coli polymerase, and by modification of the host core polymerase. The modified polymerase can initiate RNA synthesis corresponding to late bacteriophage genes. RNA polymerase isolated from T4-infected E. coli cells retains the rifampicin sensitivity of the host polymerase (8,9) and is quantitatively inhibited by antiserum against the host polymerase (10).

A quite different picture has emerged in T7-infected cells. Shortly after infection of a sensitive host, phage production becomes resistant to rifampicin (11). It is also known that late RNA synthesis depends on the function of T7 gene 1 (12). These phenomena have been explained by Chamberlin et al (13) who demonstrated that the T7 gene 1 codes for a new RNA polymerase which is insensitive to rifampicin and to antiserum against E. coli RNA polymerase. The T7 enzyme has been shown to be physically and

biochemically distinct from the host E. coli polymerase. The template specificity of the T7 enzyme is striking; the only efficient template besides T7 DNA is poly dG:dC.

The present communication describes experiments which show that as in the case of phage T7, and unlike T4, infection of E. coli B with phage T3 leads to the induction of a new RNA polymerase which is physically and biochemically distinct from E. coli polymerase. T3 polymerase is specific for its own DNA template and cannot utilize T7 DNA. Thus it is a different enzyme than the T7-induced polymerase described by Chamberlin et al (13). A preliminary report of this work has been communicated (14).

MATERIALS AND METHODS: E. coli RNA polymerase and all DNA's described in these studies were prepared as described previously (15). dAT copolymer and dG:dC hompolymer were purchased from Miles Laboratories. The growth and harvesting of T3 infected E. coli B cells were as described by Gefter (16). The multiplicity of infection was seven and infection was allowed to proceed for eight min. The sources of other reagents described in this report have been listed in previous communications from this laboratory (15,17).

Two different methods were utilized to measure RNA polymerase activity. Method A was used to measure both T3-induced and E. coli RNA polymerase. Assay Method A could be made specific for T3 polymerase by adding rifampicin (2 μg/ml) to reaction mixtures. Under these conditions, E.coli polymerase is quantitatively inhibited.

Assay Method A. Reaction mixtures (0.5 ml) contained 50 mM Tris-HCl pH 7.8, 2 mM dithiothreitol, 20 mM $MgCl_2$, 120 nmoles of each of the four ribonucleoside triphosphates, of which UTP was labeled with ^{14}C or ^{3}H (specific radioactivity = 4 x 10^6 cpm/μmole), 20 nmoles of T3 DNA and RNA polymerase. After incubation at 37° for 15 min, the reaction was terminated by the addition of 3 ml of ice cold 5% trichloroacetic acid solution. The acid-insoluble RNA product was collected by Millipore filtration as described previously (15,17). The radioactivity content of the Millipore filters was determined in toluene fluor in a liquid scintillation counter.

Assay Method B. The method is similar to Method A described above except that 3 mM of $MnCl_2$ replaced $MgCl_2$ and 40 nmoles of calf thymus DNA were used as template. This method was used for measuring host cell RNA polymerase specifically since the phage polymerase is inactive with calf thymus DNA and Mn^{++}.

Preparation of T3-induced RNA polymerase. Three gms of T3-infected

cells were ground with 6 gms of Alcoa Alumina in a pre-cooled mortar until a fine paste was obtained. The mixture was extracted with 18 ml of 0.02 M Tris-HCl buffer, pH 7.8, 0.01 M $MgCl_2$ and 2 x 10^{-3} M dithiothreitol. From this extract, a high speed supernatant (78,000 x g average) was prepared by the procedure described previously for preparation of E. coli RNA polymerase (15). T3 polymerase activity present in the supernatant fluid was further purified by phase partition in polyethylene glycol-Dextran (18) followed by chromatography on DEAE-cellulose and subsequently on phosphocellulose. Details of the purification procedure will be published elsewhere. The procedure resulted in 50-fold purification of T3-polymerase from the original crude extract, with an overall yield of about 18%. The final specific activity of the phosphocellulose fraction was 2500 units/mg of protein under the assay conditions described. In this assay, 1 unit of polymerase is equal to the incorporation of one nmole of ^{14}C-UMP after 15 min.

RESULTS: The first indication that T3-infected E. coli cells contain two distinct RNA polymerase activities was obtained when a dialyzed protein solution from an extract of T3-infected cells was applied to a DEAE-cellulose column (Figure 1). Elution with increasing concentrations of KCl resolved

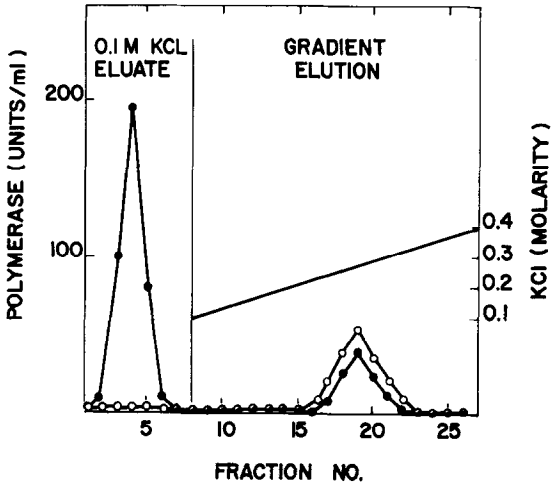

Figure 1. DEAE-cellulose chromatography of RNA polymerase activities present in T3-infected cells. A dialyzed protein fraction (30 mg) obtained from polyethylene glycol-dextran phase partitioning of a crude extract of T3-infected cells was applied to a column of DEAE-cellulose (10 ml) equilibrated with 0.05 M Tris-HCl (pH 7.8). After an initial wash of the column with 20 ml of the above buffer, the column was washed with 40 ml of buffer containing 0.1 M KCl. A linear gradient of 80 ml total volume, from 0.1 M KCl to 0.4 M KCl was then applied. All elution buffers also contained 0.05 M Tris HCl (pH 7.8), 10^{-3} M dithiothreitol and 5 x 10^{-4} M EDTA. 5 ml fractions were collected.
(●——●) ^{3}H-UMP incorporated with T3 DNA as template (Assay A).
(○——○) ^{3}H-UMP incorporated with calf thymus DNA as template (Assay B).

two peaks of RNA polymerase activity, one in the range of 0.1 M KCl (Fraction A) and another in the range of 0.28 M KCl (Fraction B). All of the RNA polymerase activitity in uninfected cells treated in the same fashion as above eluted at about 0.28 M KCl (data not shown). Further characterization of the two DEAE-cellulose fractions revealed that while Fraction A was active with T3 DNA as template and completely inactive with calf thymus DNA, Fraction B was active with both templates. The ratio of activities toward these two templates exhibited by Fraction B was identical to that obtained with purified E. coli RNA polymerase. Using T3 DNA as template, there were 10 times as many polymerase units in fraction A as in fraction B.

Further proof that fraction A and the purified phosphocellulose fraction obtained from fraction A contained a new polymerase activity was obtained by measuring the sensitivities of the fractions to known inhibitors of E. coli RNA polymerase; rifampicin and antiserum to E. coli RNA polymerase. Table 1 clearly shows that fraction B behaved as E. coli polymerase, while fraction A and the purified T3-phosphocellulose fraction were unaffected both by rifampicin and by antiserum against E. coli polymerase. In addition, while E. coli RNA polymerase was active with both Mn^{++} and Mg^{++} and unaffected by 0.1 M KCl the T3 polymerase was only active with Mg^{++} and was inhibited to the extent of approximately 70% by 0.1 M KCl.

Further proof that T3 polymerase is a protein distinct from E. coli polymerase came from zone sedimentation analysis in glycerol gradients (Figure 2). T3 polymerase sedimented much more slowly than the E. coli

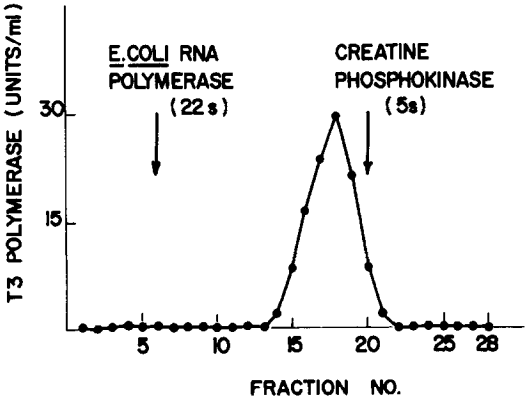

Figure 2. Zone sedimentation of T3 RNA polymerase in a glycerol gradient. Fifty units of T3 RNA polymerase (phosphocellulose fraction) were layered onto a 10-30 percent glycerol gradient (12 ml) and sedimented at 40,000 rpm for 16 hrs in an SW41 rotor. The gradient also contained 0.05 M Tris-HCl (pH 7.8) and 10^{-3} M dithiothreitol. E. coli RNA polymerase (22S) and phosphocreatine kinase (5S) were run in parallel tubes. The arrows indicate the peak positions of each.

TABLE 1. Sensitivity of E. coli and T3 Polymerases to Various Agents

	ADDITIONS	E. coli Polymerase		T3 Polymerase	
		Purified	Fraction B	Purified	Fraction A
		mμm of ^{14}C-UMP incorporated			
1.	NONE	5.1	0.8	2.2	1.8
2.	Rifampicin	0.2	0.1	2.3	1.7
3.	Antibody to E.coli Polymerase	0.3	0.1	2.3	1.6
4.	omit $MgCl_2$ and add $MnCl_2$	4.7	0.9	0.1	0.1
5.	KCl	4.8	0.8	0.6	0.5

Both the T3 polymerase (Fraction A and phosphocellulose fraction) and E. coli polymerase (Fraction B and purified enzyme - ASIII (15) were assayed by Method A. Where indicated 2 μg of rifampicin or 1 μg of antibody to E.coli RNA polymerase was added to the reaction mixtures. In line 4, 3 mM $MnCl_2$ replaced $MgCl_2$ and in line 5, KCl was added to a final concentration of 0.1 M. Amounts of polymerase added in each case were as follows: Fraction A, 1 μg; Phosphocellulose fraction, 0.1 μg; Fraction B, 3.5 μg; Purified E.coli polymerase, 1.8 μg.

TABLE 2. Template Specificity of Purified T3 Induced Polymerase

Template DNA Added	E. coli Polymerase		T3 Polymerase
	^{14}C-nucleotide incorporated		
T3	4.7		2.2
T7	4.9		0.2
T4	7.2		<0.1
Calf Thymus	3.5		<0.1
Denatured T3	1.8		0.3
dAT copolymer	9.1		<0.1
dG:dC homopolymer	4.1	(^{14}C-GMP)	4.8
	0.8	(^{14}C-CMP)	<0.1

The conditions of assay were as described in the text (Method A) except that 20 nmoles of each of the indicated DNA templates or 10 nmoles of dAT copolymer or dG:dC homopolymer replaced T3 DNA. When dG:dC was used as template, only ^{14}C-GTP or ^{14}C-CTP was added as nucleotide substrate. With dAT copolymer, only ^{14}C-UTP and ATP were added as substrates. Assays were carried out with 0.1 μg of T3 polymerase (phosphocellulose fraction) or with 1 μg of purified E. coli polymerase.

polymerase, which is known to have a sedimentation coefficient of 22S. The sedimentation coefficient for the T3 polymerase was calculated to be approximately 6.5 S.

T3 polymerase shows a striking template specificity (Table 2). While the E. coli polymerase was active with all the native DNA templates tested, T3 polymerase was active only with T3 DNA. Denatured T3 was also active but with greatly reduced efficiency. The T3 enzyme was inactive with dAT copolymer, but copied the homopolymer dG:dC quite efficiently, yielding poly rG exclusively. Poly rC was not synthesized at all, showing that the enzyme does not recognize the dG strand.

Although not shown in Table 2, the formation of RNA product by T3 polymerase required the presence of all four ribonucleoside triphosphates, Mg^{++} (20 mM optimal concentration) and T3 DNA template. Omission of any one of the above components from the reaction mixture led to no incorporation of nucleotides into an acid-insoluble RNA product (<0.1 nmole). The inclusion of pancreatic DNase or RNase in the reaction mixture led to no detectable (<0.1 nmole) of acid-insoluble radioactivity. Furthermore, the acid-insoluble RNA product formed in reaction mixture was rendered acid soluble by (1) incubation of the product with pancreatic RNase (1 μg) for 5 min at 37° or (2) incubation in 0.3 N NaOH for 18 hours at 37°.

The time course of appearance of T3 polymerase in E. coli B cells infected with phage T3 was also investigated. T3-specific polymerase was detectable within four min after infection and was maximal by eight min (Fig 3). Addition of chloramphenicol (50 μg/ml) to the bacterial cultures at the time of infection resulted in no detectable induction of a new polymerase activity. Induction of the T3 polymerase activity thus requires de novo protein synthesis presumably directed by the genetic information of the infecting particle. The level of E. coli RNA polymerase activity measured by assay Method B remained constant during the infective cycle (data not shown).

DISCUSSION: The above results clearly indicate that after infection of E. coli B with phage T3, a new DNA dependent RNA polymerase is induced. The situation is thus analogous to the induction of a new RNA polymerase in T7-infected cells as described by Chamberlin et al (13). The T3 and T7 induced polymerases have very similar biochemical properties. Both polymerases work efficiently with the homopolymer pair dG:dC, making poly rG exclusively. This suggests that the promoter sites recognized by these two polymerases may be C-rich regions of the DNA. The T3 induced polymerase appears to be distinct from T7 polymerase since, unlike the latter, the T3 polymerase is not active with T7 DNA as template. Finally, the T3-induced polymerase may be involved in transcription of late mRNA formed in T3-infected cells.

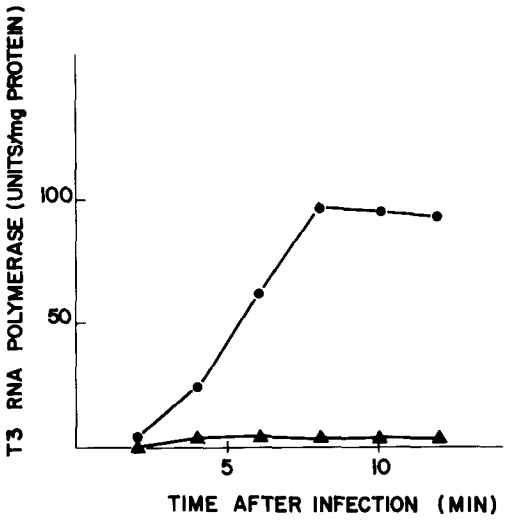

Figure 3. Induction of T3 RNA polymerase activity after T3 bacteriophage infection of E. coli B. E. coli B was grown at 37° with virogous aeration in 4 l. of casamino acid medium as described by Gefter (16) supplemented with 0.1% yeast extract. When the cell density reached 8 x 10^8 cells/ml, the culture was infected with phage T3 at a multiplicity of infection of 7 phage particles/bacteria. Aliquots (400 ml) were taken at 2 min intervals and were immediately poured into crushed ice. The cells were harvested by centrifugation and were stored frozen at -20°. Cell free extracts (78,000 x g supernatant) were prepared as described in 'Methods'. RNA polymerase activity was measured by assay Method A in the presence of 2 μg/ml rifampicin.
(●——●)no chloramphenicol; (▲——▲) with chloramphenicol.
Under the above conditions of infection, lysis was complete in 16 min.

Studies to evaluate this hypothesis and further biochemical characterization of this new phage polymerase are in progress.

ACKNOWLEDGEMENTS: This work was supported by grants from the National Institutes of Health, the American Heart Association, Inc. and the Life Insurance Medical Research Fund. The author is an Established Investigator of the American Heart Association.

REFERENCES:

1. Geiduschek, E.P., and Haselkorn, R., Ann. Rev. Biochem., 38, 647 (1969).
2. Travers, A., Nature, 229, 69 (1971).
3. Travers, A., Nature, 225, 1009 (1970).
4. Walter, G., Seifert, W., and Zillig, W., Biochem. Biophys. Res. Commun., 30, 240 (1968).
5. Goff, C.G. and Weber, K., Cold Spring Harb. Symp. Quant. Biol., 35, 101 (1970)
6. Travers, A., Cold Spring Harb. Symp. Quant. Biol., 35, 241 (1970).
7. Stevens, A., Biochem. Biophys. Res. Commun., 41, 367 (1970).
8. Takeda, Y., Oyama, Y., Nakajima, K., and Yura, T., Biochem. Biophys. Res. Commun., 36, 533 (1969).

9. Haselkorn, R., Vogel, M. and Brown, R., Nature, 221, 836 (1969).
10. Martin, G.S. and Tocchini-Valentini, G.P. in Martin, G.S., Thesis, University of Cambridge, England (1968).
11. Summers, W.C., and Siegel, R.B., Nature, 223, 1111 (1969).
12. Siegel, R.B., and Summers, W.C., J. Mol. Biol. 49, 115 (1970).
13. Chamberlin, M., McGrath, J., and Waskell, L., Nature, 228, 227 (1970).
14. Lockwood, A.L., Huang, H., and Maitra, U., Abstracts, Federation Proceedings, 1971, in press.
15. Maitra, U. and Hurwitz, J., J. Biol. Chem. 242, 4908 (1967).
16. Gefter, M., Ph.D. Thesis, Albert Einstein College of Medicine of Yeshiva University, June, 1967.
17. Maitra, U. and Barash, F., Proc. Natl. Acad. Sci., 64, 729 (1969).
18. Okazaki, T., and Kornberg, A., J. Biol. Chem., 239, 259 (1969).

ERRATA

Volume 42, No. 3, 1971, in the Communication, "The Molecular Structure of Firefly D-(-)-Luciferin : A Single Crystal X-ray Analysis", by G. E. Blank, J. Pletcher, and M. Sax; pages 583-588, Page 584 - Fig. 1 - bond $C_{8'}-C_{9'}$ should be labelled: "1.379 Å".

Volume 42, No. 6, 1971, in the Communication, "The Origin of Light and Heavy Chains of Immunoglobulins"' by M. S. Kanungo; Pages 1176-1179, Page 1176, line 7 of first paragraph should read 321 instead of 328; Legend for Figure 1 - should read "Mechanism of origin of L and H chains of Ig. v, variable; c, common". The following line should be added to the text: This research was supported by PL-480 Project No. A7-AH-57.