*THE STRUCTURAL GENE FOR DEOXYRIBONUCLEIC ACID POLYMERASE IN BACTERIOPHAGES T4 AND T5**

By A. de Waard,† A. V. Paul, and I. R. Lehman

DEPARTMENT OF BIOCHEMISTRY, STANFORD UNIVERSITY SCHOOL OF MEDICINE

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Genetic studies by Epstein, Edgar, and their colleagues[1-3] with conditional lethal (*amber* and temperature-sensitive) mutants of bacteriophage T4 have led to the construction of a genetic map for this phage, containing approximately 70 different genes of which 20 are believed to function in DNA synthesis. Thus far, only one of these genes has been identified with a specific enzymatic function. Wiberg *et al.*[4] investigated seven *amber* mutants; five of these, representing genes 39, 41, 42, 44, and 46, were defective in DNA synthesis. Of these, mutants in gene 42 were found incapable of inducing the enzyme deoxycytidylate hydroxymethylase in the restrictive host (*Escherichia coli* B). Subsequent work by Dirksen *et al.*[5] suggested that gene 42 is the structural gene for this enzyme. Mutants of gene 43 were not investigated in these studies.

Dr. Edgar generously made available to us a series of *amber* and temperature-sensitive mutants of bacteriophage T4 and we have examined them for their ability to induce the T4 DNA polymerase.[6] These studies have led to the identification of gene 43 as the structural gene for the phage-induced polymerase. After this work was completed, Dr. H. R. Warner, of the Department of Biochemistry of the University of Minnesota, informed us that he had independently identified gene 43 with the T4 DNA polymerase.

We have also examined four temperature-sensitive mutants of bacteriophage T5,[7] kindly provided by Dr. Frank Lanni, for their ability to induce the T5 DNA polymerase.[8] One of these was found to induce the synthesis of a DNA polymerase of significantly greater heat-lability than that induced by the wild-type phage. The cistron bearing this mutation would therefore appear to be the structural gene for the T5-induced DNA polymerase.

Materials and Methods.—Materials: P^{32}-labeled dTTP[9] and dATP were prepared as described previously, or purchased from the International Chemical and Nuclear Corporation, City of Industry, Calif. Unlabeled nucleotides and salmon sperm DNA were purchased from the California Foundation for Biochemical Research, Los Angeles, Calif. C^{14}-labeled UDPG (specific activity 20 μc/μmole) was prepared according to Kornberg, Zimmerman, and Kornberg.[10] Antiserum to *E. coli* DNA polymerase was prepared as described by Aposhian and Kornberg.[6] DNA polymerase from *E. coli* B infected with T4 *am* N82 was purified by the procedure of Lucas and Kornberg.[11] The purified wild-type T5-induced DNA polymerase[8] was generously provided by Dr. Maurice J. Bessman.

Growth of bacteriophages and preparation of cell extracts: The T4 bacteriophages and *E. coli* strains B, B/5, and CR 63 were provided by Dr. R. S. Edgar. Media used for the propagation and assay of the T4 phages were those described by Edgar and Lielausis.[3] *E. coli* strain F and T5 mutants *ts* 34, *ts* 51, *ts* 52, and *ts* 53[7] were provided by Dr. Frank Lanni. Mutants *ts* 51, *ts* 52, and *ts* 53 were isolated by Dr. Ling Chu in Dr. Lanni's laboratory.

Stocks of T4 *amber* mutants were prepared by infecting *E. coli* CR 63 (the permissive host) in H broth[3] at a multiplicity of infection of 0.1. The cultures were grown at 37° under forced aeration until lysis occurred after approximately 4 hr. A few drops of chloroform were added and after 5 min the lysates were chilled to 0° and cleared of debris by centrifugation for 10 min at 9,000 × *g*.

Extracts of T4 *amber*-infected cells of the restrictive host were made in the following way: cultures of *E. coli* B/5 (40 ml) were grown at 37° to a density of 5×10^8 cells/ml, and T4 bacteriophage was added at a multiplicity of 5. After 20 min the suspension was poured onto crushed ice. The infected cells were harvested at 0°, washed once with cold 0.05 *M* Tris-Cl buffer, pH 7.5, containing 0.002 *M* glutathione and 0.002 *M* EDTA. The pellets were resuspended in 13 ml of the same buffer without EDTA, and the cells were disrupted by sonic irradiation for 2 min with a Branson S75 sonifier. The cell debris was removed by centrifugation for 10 min at 9,000 × *g*. Extracts prepared in this way contained 0.7–0.9 mg protein/ml.

Stocks of temperature-sensitive T4 mutants were grown at 25° on *E. coli* B/5 at a multiplicity of infection of 0.1 for approximately 6 hr until heavy foaming indicated lysis. The lysates were chilled to 0° and cleared of debris by centrifugation for 10 min at 9,000 × *g*. In experiments in which the effect of temperature on enzyme formation was studied, *E. coli* B/5 was infected with phage at 37° at a multiplicity of 5. One half of the infected culture was immediately cooled to 25° and the remainder was kept at 37°. After 20 min the infected cells were chilled and extracts prepared as above.

Stocks of T5 were prepared in the following way: a culture of *E. coli* B (50 liters) was grown at 37° under forced aeration in a modified M-9 medium[10] to a cell density of 2×10^8/ml. T5 bacteriophage was added at a multiplicity of 1 and aeration was continued for about 3 hr until lysis occurred. The lysate was passed through a Beckman model 170 continuous-flow centrifuge first to remove the cell debris, and then to harvest the phage. The pellet was taken up in 0.02 *M* potassium phosphate buffer, pH 7.0, containing 0.15 *M* NaCl. Stocks of *ts* 53, the temperature-sensitive mutant, were made in the same way, except that *E. coli* F was used as the host and infection was carried out in 25-liter cultures at 30°.

For the preparation of T5 phage-infected extracts, *E. coli* F was grown in MGM medium containing 10^{-3} *M* $CaCl_2$[12] with aeration to a cell density of 2×10^8/ml. T5 bacteriophage was added at a multiplicity of 4 and after 30 min at 30° or 43° the culture (200 ml) was poured onto crushed ice. The cells were harvested by centrifugation for 10 min at 7,000 × *g*, then resuspended in 2 ml of 0.05 *M* glycylglycine buffer, pH 7.0, containing 0.001 *M* glutathione. The suspension was stored at −15° for 1 day and the cells were disrupted by sonic irradiation for 1 min with a Mullard sonicator. Cell debris was removed by centrifugation for 10 min at 9,000 × *g*, yielding extracts that contained about 3 mg protein/ml.

Enzyme assays: Assay of T4 DNA polymerase was similar to that described by Aposhian and Kornberg,[6] except that EDTA was omitted from the reaction mixture and 0.2 μmole of KF was added together with an amount of antiserum to *E. coli* DNA polymerase which was sufficient to inactivate 80–90% of the polymerase activity present in the aliquots of uninfected extract used. The reaction was terminated after incubation for 30 min at the indicated temperature, and the acid-insoluble radioactivity was determined by the glass filter paper assay as described by Richardson *et al.*[13] Radioactivity was measured with a Packard Tricarb liquid scintillation spectrometer. One unit of enzyme is defined as the amount catalyzing the incorporation of 10 mμmoles of P^{32}-labeled deoxynucleoside triphosphate into an acid-insoluble product in 30 min.

The assay for T5 DNA polymerase was similar to that described by Orr, Herriott, and Bessman.[8] The reaction mixture (0.3 ml) contained 20 μmoles of Tris, pH 8.6, 2 μmoles of $MgCl_2$, 10 μmoles of β-mercaptoethanol, 250 mμmoles of heat-denatured salmon sperm DNA (nucleotide), 60 μmoles of NH_4Cl, 25 mμmoles each of dGTP, dCTP, and dTTP, 15 mμmoles of $dATP^{32}$, antiserum to *E. coli* DNA polymerase, and 0.008–0.05 unit of enzyme. The amount of antiserum used was sufficient to inactivate 80–90% of the DNA polymerase activity present in the aliquots of the uninfected cell extracts used. After incubation for 20 min at 25° or 43°, the acid-insoluble radioactivity was determined according to Lehman *et al.*[14] Radioactivity was measured with a Nuclear-Chicago model 186 counter equipped with a Micromil window.

One unit of enzyme is defined as the amount catalyzing the incorporation of 10 mμmoles of $dATP^{32}$ into an acid-insoluble product in 20 min at 25°.

Assay of HMC-β-glucosyl transferase was according to Josse and Kornberg.[15]

Protein was determined by the method of Lowry *et al.*[16] or, in the case of dilute samples (less than 20 μg/ml), by the method of Waddell.[17]

Results.—T4 amber mutants: The initial screening was facilitated by the availability of several multicistronic mutants which had been constructed for this pur-

pose by Dr. R. S. Edgar. Of the mutants tested, only X5, which bears mutations in genes 41, 42, 43, 44, and 45, was found to be defective in the synthesis of DNA polymerase (Table 1).[18] The level of polymerase activity in extracts of cells infected with this phage was the same as that found in extracts of uninfected cells. Antiserum to *E. coli* DNA polymerase was included in the reaction mixtures so that the values recorded for the X5-infected and uninfected cell extracts probably represent *E. coli* DNA polymerase activity which has not been neutralized by the antiserum.

The absence of phage-induced DNA polymerase activity was not due to the inability of the X5 mutant to infect *E. coli* B, since extracts of X5-infected cells showed normal levels of HMC-β-glucosyl transferase activity (0.04 unit/mg protein).

TABLE 1

T4 DNA POLYMERASE LEVELS IN EXTRACTS OF *E. coli* B INFECTED WITH MULTICISTRONIC T4 *amber* MUTANTS

Mutant	DNA polymerase activity (mμmoles dTMP^{32} incorporated/mg protein)
Uninfected cells	0.75
T4 D *am*$^+$ (wild type)	9.0
X2*n*	15.0
X2*m*	16.2
X2*f*	16.5
X5	0.75
N122/*m*	3.50
m	2.25
N122*	11.2

* The discrepancy between the result shown for *am* N122 and that reported by Wiberg *et al.*[4] might be explained in the following way. The *am* N122 examined by Wiberg *et al.* has recently been recognized by Flatgaard and Edgar[23] to be a double mutant containing the N122 (gene 42) mutation and a second mutation designated *m*. As indicated, *am* N122 is not defective in DNA polymerase synthesis; instead, mutation *m*, physically close to mutation N122, is apparently responsible for the low level of DNA polymerase observed in the double mutant N122/*m*.

DNA polymerase assays were carried out at 37° as described under *Materials and Methods*, using α-labeled dTTP^{32} (7.5 $\times$ 10^6 cpm/μmole). The multiple mutants were composed of the following single mutants: X2*n*, BL292 (gene 55) and E727 (gene 49); X2*m*, N130 (gene 46) and A456 (gene 47); X2*f*, A453 (gene 32) and N132 (gene 33); X5, N81 (gene 41), N122 (gene 42), B22 (gene 44), and E10 (gene 45).

Single mutants representing genes in which X5 is defective were tested for their ability to induce the T4 DNA polymerase. Again the success of infection was monitored by assays of HMC-β-glucosyl transferase activity. As shown in Table 2, only *am* N101 (gene 43) of the mutants tested failed to induce the T4 polymerase.

Since mixed infection with an *amber* mutant and wild-type phage results in phage production,[2] it is unlikely that the lack of T4 DNA polymerase activity is due to an inhibitor produced after infection by the mutant phage. Moreover, when extracts of *am* N81- and *am* N101-infected cells were mixed and the mixture assayed, the DNA polymerase activities were completely additive (0.41 unit observed as compared with an expected activity of 0.38 unit) indicating that extracts of *am* N101-infected cells do not contain an excess of a DNA polymerase inhibitor.

TABLE 2

T4 DNA POLYMERASE AND HMC-β-GLUCOSYL TRANSFERASE ACTIVITIES INDUCED BY MUTANTS REPRESENTING GENES 41, 43, 44, AND 45

Mutant	Gene	DNA polymerase activity (mμmoles dTMP^{32} incorporated/mg protein)	β-Glucosyl transferase activity (mμmoles C^{14}-glucose incorporated/mg protein)
T4 D *am*$^+$ (wild type)			13.6
am N81	41	5.6	19.9
am N101	43	0.47	7.1
am N82	44	3.3	8.1
am E10	45	2.7	—
ts L159*	45	4.2	15.8
Uninfected cells		0.62	<0.3†

* A temperature-sensitive mutant which produces no phage when *E. coli* B/5 is infected at 39.5°.
† This value is below the level of significance for this assay.
DNA polymerase and β-glucosyl transferase assays were performed at 37° as described under *Materials and Methods*. The specific activity of the α-labeled dTTP^{32} was 5.0 $\times$ 10^6 cpm/μmole.

TABLE 3

EFFECT OF TEMPERATURE ON DNA POLYMERASE ACTIVITY INDUCED BY TEMPERATURE-SENSITIVE MUTANTS OF PHAGE T4

Mutant	Temperature of infection	DNA Polymerase Activity ($m\mu$moles $dAMP^{32}$ incorporated) 25°	37°	$Q^{37°}_{25°}$
Control				
T4 D *am*+*	25°	0.11	0.28	2.5
	37°	0.05	0.13	2.6
ts L159 (gene 45)	25°	0.19	0.46	2.4
	37°	0.15	0.39	2.6
Group I				
ts L141	25°	0.16	0.29	1.8
	37°	0.25	0.41	1.6
ts S9	25°	0.21	0.28	1.3
	37°	0.11	0.16	1.5
Group II				
ts L97	25°	0.16	0.44	2.7
	37°	<0.02	0.02	—
ts L107	25°	0.11	0.20	1.8
	37°	<0.02	<0.02	
Group III				
ts L91	25°	0.21	0.07	0.34
	37°	0.22	0.09	0.41

* The activity of the T4 D *am*+-induced DNA polymerase observed in this experiment was considerably below that usually found; see, for example, Table 4.

DNA polymerase assays were performed as described under *Materials and Methods* except that the time of incubation was 20 min and the temperature was 25° or 37° as indicated. The labeled substrate was $dATP^{32}$ (6.3×10^6 cpm/μmole). The values are corrected for the level of incorporation found in the uninfected extract. $Q^{37°}_{25°}$ refers to the ratio of activities at 37° and at 25°.

Temperature-sensitive mutants of T4: The DNA polymerase activities induced by five temperature-sensitive mutants of bacteriophage T4 known to map in gene 43 were examined for temperature-sensitivity.

Extracts were prepared of cells infected with T4 phages designated by Edgar as *ts* L97, *ts* L107, *ts* L141, *ts* S9, and *ts* L91.[1] One series of infections was at 25° and a second was at 37°. The extracts were then tested for their T4 DNA polymerase activity at 25° and at 37° (Table 3).

Among the five temperature-sensitive mutants examined, three types of response could be distinguished. The first is typified by the phages of Group I, which did not differ significantly from the wild type. In this case, active enzyme was synthesized both at 37° and at 25°, and on incubation *in vitro* the enzyme was more active at 37° than at 25°. A second type of temperature response is shown by the mutants of Group II which produced enzyme at 25° but not at 37°. Assays of HMC-β-glucosyl transferase activity indicated that infection at 37° proceeded normally. In this case, however, extracts of cells infected at 25° showed a significantly higher level of DNA polymerase activity at 37° than at 25°. The third type of response is that given by phage *ts* L91 (Group III) in which equivalent levels of enzyme were produced at 37° and at 25°; however, activity measured at 25° was nearly twofold greater than at 37°.

To determine whether the greater heat-lability of the polymerase induced by T4 *ts* L91 was due to a change in the primary structure of the enzyme itself or to an indirect effect such as, for example, the induction of a heat-activated deoxyribonuclease, the DNA polymerase was partially purified (approximately 60-fold) from extracts of *E. coli* B infected with phage *ts* L91.[19] The $Q^{37°}_{25°}$ (ratio of activity at 37° to that at 25°) of the DNA polymerase activity in the partially purified enzyme preparation was found to be very similar to that observed in the crude extract. Moreover, $Q^{37°}_{25°}$ remained essentially constant (approximately 0.5) through the three purification steps used in the procedure (Table 4). On the other hand, a partially purified DNA polymerase preparation derived from *E. coli* B cells infected with phage T4 *am* N82 (gene 44) showed a $Q^{37°}_{25°}$ of 2.8.

The more rapid denaturation at 37° of the partially purified *ts* L91 as compared with the *am* N82 DNA polymerase was observed by preincubating the two enzymes at 37° and then assaying the remaining activity at 25°. As shown in Figure 1, the *ts* L91-induced enzyme was inactivated at 37° at a significantly greater rate than the *am* N82 polymerase.

The partially purified preparations of *ts* L91 and *am* N82 DNA polymerase contained significant levels of the T4-induced oligonucleotide diesterase activity of the type found by Oleson and Koerner[20] in T2-infected *E. coli* extracts (610 and 2475 units/mg protein, respectively, when assayed at 37°). As a further test of the possibility that this activity in some way might be related to the increased temperature-sensitivity of the *ts* L91 enzyme, both the *ts* L91 and the *am* N82 enzymes were assayed for oligonucleotide diesterase activity at 25° and at 37°. The $Q_{25°}^{37°}$ values observed were identical (1.4).

TABLE 4

RELATIVE HEAT-LABILITIES OF PARTIALLY PURIFIED T4 *ts* L91 AND T4 *am* N82 DNA POLYMERASES

Fraction	DNA Polymerase Activity (units/mg protein) Assayed at 25°	Assayed at 37°	$Q_{25°}^{37°}$
ts L91 DNA polymerase:			
Extract*	0.8	0.4	0.50
Gel filtration	2.3	0.9	0.40
DEAE-cellulose	9.0	3.5	0.39
Phosphocellulose	50.0	26.0	0.52
am N82 DNA polymerase:			
DEAE-cellulose	87.0	240.00	2.8

* The specific activity of an extract of cells infected at 37° with T4 D am^+ (wild type) was 2.4 units/mg when assayed at 25°, and 4.7 units/mg protein when assayed at 37°.
DNA polymerase assays were performed as described under *Materials and Methods* with the exception that the time of incubation was 20 min and the temperature was 25° or 37° as indicated. The labeled substrate was $dATP^{32}$ (4.0×10^6 cpm/μmole). $Q_{25°}^{37°}$ refers to the ratio of activities at 37° and 25°.

Temperature-sensitive mutants of T5: Extracts of *E. coli* F infected with four temperature-sensitive mutants of phage T5 (*ts* 34, *ts* 51, *ts* 52, and *ts* 53) were also examined for their phage-induced DNA polymerase activity. Of these, only the polymerase induced by T5 *ts* 53 appeared to be more heat-labile than the enzyme induced by the wild-type phage. As indicated in Table 5, the temperature response of the *ts* 53 DNA polymerase was qualitatively similar to that induced by the *ts* L91 mutant of T4. That is, infection at either 30° or 43° resulted in the induction of a DNA polymerase whose activity, in contrast to the enzyme induced by the wild-type phage, was less when assayed at 43° than at 25°.

The T5-induced DNA polymerase was purified approximately 60-fold from extracts of *E. coli* F infected at 30° with phage *ts* 53.[21] The $Q_{25°}^{43°}$ (ratio of activity at 43° to that at 25°) of the partially purified *ts* 53 polymerase was 0.04 at 20 min compared with a value of 1.4 for the wild-type T5-induced enzyme (Fig. 2).

TABLE 5

EFFECT OF TEMPERATURE ON DNA POLYMERASE ACTIVITY INDUCED BY A TEMPERATURE-SENSITIVE MUTANT OF PHAGE T5

Phage	Temperature of infection	DNA Polymerase Activity (mμmoles $dAMP^{32}$-incorporated) 25°	43°	$Q_{25°}^{43°}$
T5 *ts* 53	30°	0.081	0.030	0.37
	43°	0.039	0.024	0.61
T5 wild type	30°	0.295	0.306	1.03
	43°	0.335	0.348	1.04

T5 DNA polymerase activities were measured as described under *Materials and Methods* except that the incubations were for 30 min at 25° or 43°. The labeled substrate was $dATP^{32}$ (4.0×10^6 cpm/μmole). The values have been corrected for a low level of incorporation found with the uninfected cell extracts in the presence of the antiserum to *E. coli* DNA polymerase. $Q_{25°}^{43°}$ refers to the ratio of activities at 43° and at 25°.

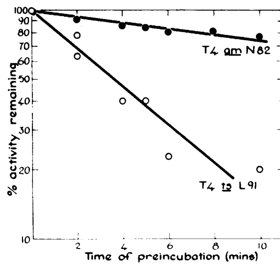

FIG. 1.—Effect of preincubation (37°) on the enzymatic activity at 25° of partially purified T4 *am* N82 DNA polymerase (DEAE-cellulose fraction[11]) and T4 *ts* L91 DNA polymerase (DEAE-cellulose fraction[19]). The enzymes were diluted in 0.05 *M* TrisCl buffer, pH 7.5, containing 0.12 *M* $(NH_4)_2SO_4$ and 0.5 mg crystalline bovine plasma albumin/ml, and incubated at 37°. At the times indicated, samples were withdrawn and added to precooled assay mixtures. After standing at 0° for 5 min, the assays were run at 25°. Incubation was for 20 min. The labeled substrate was α-labeled $dATP^{32}$ (3.35 × 10^6 cpm/μmole).

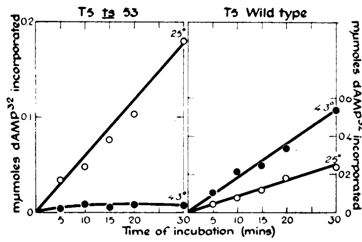

FIG. 2.—Effect of temperature on the activity of *ts* 53 and wild-type T5 DNA polymerases. A series of reaction mixtures was set up with either the *ts* 53 DNA polymerase or with the wild-type T5 DNA polymerase. The *ts* 53 DNA polymerase was the phosphocellulose fraction, specific activity 59; wild-type T5 DNA polymerase was an aged DEAE-cellulose fraction that originally had a specific activity of 430, but whose activity had fallen to a value of 10. The tubes were incubated at 25° or 43° for the indicated times. $dATP^{32}$ (4.2 × 10^6 cpm/μmole) incorporation into acid-insoluble material was measured as described under *Materials and Methods.*

The rapid inactivation of the *ts* 53 DNA polymerase at 43° appeared to be partially reversible. After preincubation of the enzyme at 43° for periods as long as 1 hr, subsequent assay of the enzyme at 25° indicated that approximately 70 per cent of the initial activity could still be recovered.

Discussion.—The observation that T4 *am* N101, with a mutation in gene 43, does not produce an active T4 DNA polymerase in *E. coli* B (the nonpermissive host) is compatible with the idea that gene 43 functions in the synthesis of this enzyme. Moreover, the isolation of the altered enzyme from cells infected with *ts* L91, which also bears a mutation in gene 43, and the demonstration of a heat-lability greater than that of the wild-type polymerase suggests that this gene is indeed the structural gene for the T4-induced polymerase. Similarly, the identification of a highly temperature-sensitive DNA polymerase induced by a temperature-sensitive mutant of phage T5 suggests that the gene bearing the mutation is the structural gene for the T5-induced DNA polymerase. Since a single-step mutation in phage T4 or T5 leads to defective DNA synthesis *in vivo*[1, 24] and the only demonstrable enzymatic defect is that in DNA polymerase, the implication is clear that the DNA polymerase induced by phage infection and measured *in vitro* is, in part at least, responsible for synthesis of the phage DNA *in vivo.* Of interest in this regard is the additional implication that the host *E. coli* DNA polymerase, whose level remains unchanged during the course of infection,[22] is incapable of catalyzing phage DNA synthesis.

The three groups of temperature-sensitive mutants of T4 observed deserve some comment. In the first group the enzymatic lesion is not detected by *in vitro* assays at 25° or 37°. The lesion, however, prevents phage development *in vivo* at 37°. It is not known why the *in vitro* assays do not detect it; assays at higher temperatures or at other pH's might do so. In the second group of mutants, a functional enzyme is not formed at 37° but once formed at 25° it is stable at 37°. Again the reason for this behavior is not known. One possibility is that there is a thermolabile step in the formation of the tertiary structure of the

protein so that a functional protein is formed at 25° but not at 37°. However, once a functional protein has been synthesized, it might then be active at 37°. The third type of T4 mutant (*ts* L91) and the analogous T5 mutant (*ts* 53) produces an enzyme which is stable at 25° but not at 37° or 43°, relative to the wild type. The finding that active enzyme is produced after infection at 25°, 37°, and 43° may be due in part to the reversibility of the inactivation noted, particularly with the polymerase induced by the temperature-sensitive mutant of phage T5.

Summary.—(1) T4 *amber* mutants known to be defective in DNA synthesis were screened for their ability to induce the T4 DNA polymerase. Of the mutants examined, only those which are associated with gene 43 were found to be defective in the synthesis of this enzyme. (2) A polymerase with greater heat-lability than that induced by wild-type T4 was partially purified from cells of *E. coli* which had been infected with a temperature-sensitive mutant, *ts* L91, known to map in gene 43. (3) A DNA polymerase, which was relatively more heat-labile than the enzyme induced by the wild-type phage, was also purified from cells of *E. coli* infected with a temperature-sensitive mutant (*ts* 53) of T5.

These findings indicate that gene 43 represents the structural gene for the T4 DNA polymerase and that the gene in which mutant *ts* 53 is defective is the structural gene for the T5 DNA polymerase. They also imply that the DNA polymerases induced upon infection with phages T4 and T5 and measured *in vitro* are, in part at least, responsible for the synthesis of phage DNA *in vivo*.

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[1] Epstein, R. H., A. Bolle, C. M. Steinberg, E. Kellenberger, E. Boy de la Tour, R. Chevalley, R. S. Edgar, M. Sussman, G. H. Denhard, and A. Lielausis, in *Synthesis and Structure of Macromolecules*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 28 (1963), p. 375.

[2] Edgar, R. S., G. H. Denhardt, and R. H. Epstein, *Genetics*, **49**, 635 (1964).

[3] Edgar, R. S., and A. Lielausis, *Genetics*, **49**, 649 (1964).

[4] Wiberg, J. S., M. L. Dirksen, R. H. Epstein, S. E. Luria, and J. M. Buchanan, these Proceedings, **48**, 293 (1962).

[5] Dirksen, M. L., J. C. Hutson, and J. M. Buchanan, these Proceedings, **50**, 507 (1963).

[6] Aposhian, H. V., and A. Kornberg, *J. Biol. Chem.*, **237**, 519 (1962).

[7] Fattig, W. D., and F. Lanni, *Genetics*, **51**, 157 (1965).

[8] Bessman, M. J., C. W. M. Orr, and S. T. Herriott, *Federation Proc.*, **24**, 286 (1965); Orr, C. W. M., S. T. Herriott, and M. J. Bessman, *J. Biol. Chem.*, in press.

[9] The following abbreviations are used: dCMP, deoxycytidine 5'-monophosphate; dTTP, dATP, dCTP, and dGTP, the deoxyribonucleoside triphosphates of deoxythymidine, deoxyadenosine, deoxycytidine, and deoxyguanosine, respectively; HMC, 5-hydroxymethylcytosine; UDPG, uridine diphosphate glucose; EDTA, ethylenediaminetetraacetate; DEAE-cellulose, diethylaminoethyl cellulose.

[10] Kornberg, S. R., S. B. Zimmerman, and A. Kornberg, *J. Biol. Chem.*, **236**, 1487 (1961).

[11] Lucas, Z., *Federation Proc.*, **24**, 286 (1965); Lucas, Z., and A. Kornberg, personal communication.

[12] Lanni, Y. T., *Virology*, **15**, 127 (1961).

[13] Richardson, C. C., C. L. Schildkraut, H. V. Aposhian, and A. Kornberg, *J. Biol. Chem.*, **239**, 242 (1964).

[14] Lehman, I. R., M. J. Bessman, E. S. Simms, and A. Kornberg, *J. Biol. Chem.*, **233**, 165 (1958).

[15] Josse, J., and A. Kornberg, *J. Biol. Chem.*, **237**, 1968 (1962).

[16] Lowry, O. H., N. L. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

[17] Waddell, W. J., *J. Lab. Clin. Med.*, **48**, 311 (1956).

[18] Extracts of *E. coli* infected with the single *amber* mutants—B24 (gene 1), C5 (gene 59), N116 (gene 39), HL626 (gene 60), H17 (gene 52), HL627 (gene 58), and N55 (gene 42)—were examined and found to contain normal levels of T4 DNA polymerase. In a single experiment, infection with *am* HL628 (gene 59) resulted in a low level of DNA polymerase activity (1.7 mμmoles of $dTMP^{32}$ incorporated/mg protein). However, in this case the HMC-β-glucosyl transferase was also abnormally low, and it is not clear whether infection was successful. A temperature-sensitive mutant, *ts* A90, which maps in gene 56 gave normal polymerase levels when assayed at 37°.

[19] The method used in the partial purification of the *ts* L91 DNA polymerase was an adaptation of procedures developed by Kornberg and his colleagues for the purification of DNA polymerases from T4 *am* N82-infected *E. coli* and from *B. subtilis* [Lucas, Z., and A. Kornberg, personal communication; Okazaki, T., and A. Kornberg, *J. Biol. Chem.*, **239**, 259 (1964)]. Cells of *E. coli* B (5×10^8/ml) were infected at 25° with *ts* L91 at a multiplicity of 3. After 20 min they were harvested as described under *Materials and Methods* and disrupted by blending with glass beads. The extract obtained (80 ml) was subjected to phase separation using dextran and polyethylene glycol. The polyethylene glycol phase was passed through a column of Sephadex G-100. The Sephadex fractions were first chromatographed on DEAE-cellulose using a gradient (300 ml) of potassium phosphate, pH 6.5, from 0.02 *M* to 0.30 *M*. The DEAE-cellulose eluates were then chromatographed on phosphocellulose, using a gradient (150 ml) of potassium phosphate, pH 6.5 from 0.1 *M* to 0.4 *M*. The *ts* L91 DNA polymerase was eluted midway in the gradient and was concentrated by dialysis against 30% polyethylene glycol. β-Mercaptoethanol (0.01 *M*) and 20% glycerol were present throughout the purification procedure.

[20] Oleson, A. E., and J. F. Koerner, *J. Biol. Chem.*, **239**, 2935 (1964).

[21] The T5 *ts* 53 DNA polymerase was purified by a modification of the method devised by Orr, Herriott, and Bessman for purification of the wild-type T5 DNA polymerase (*J. Biol. Chem.*, in press). We are grateful to Dr. Bessman for providing us with the details of this method in advance of its publication. *E. coli* (30 liters) was grown at 30° with vigorous aeration in nutrient broth containing 0.001 *M* $CaCl_2$. The cells (5×10^8/ml) were infected with *ts* 53 at a multiplicity of 5 and aeration was continued for 40 min. The cells were then harvested by means of a continuous-flow centrifuge and disrupted by sonic irradiation for 15 min using a Raytheon sonicator. The extracts were centrifuged for 3 hr at 144,000 × *g* and the supernatant fluid was recovered. To remove the nucleic acids, 90 ml of the extract (4 mg protein/ml) were subjected to phase separation using dextran and polyethylene glycol. The polyethylene glycol layer was treated with 0.1 vol of 1 *M* potassium acetate buffer, pH 4.5. The precipitate which formed was dissolved in 0.02 *M* potassium phosphate buffer, pH 7.4, containing 0.001 *M* β-mercaptoethanol, 0.0001 *M* EDTA, and 20% glycerol, and chromatographed on DEAE-cellulose using a gradient (320 ml) of 0.1 *M* NaCl to 0.6 NaCl in 0.02 *M* potassium phosphate buffer, pH 7.4. The active enzyme fractions were then chromatographed on phosphocellulose using a gradient (160 ml) of 0.1 *M* NaCl to 0.6 *M* NaCl in 0.02 *M* potassium phosphate buffer, pH 6.5. β-Mercaptoethanol (0.001 *M*), EDTA (0.0001 *M*), and 20% glycerol were present throughout all the chromatographic steps. The enzyme isolated in this way was approximately 60-fold purified over the cell extract.

[22] Josse, J., and A. Kornberg, personal communication.

[23] Flatgaard, J., and R. S. Edgar, personal communication.

[24] Paul, A. V., and I. R. Lehman, unpublished observations.