JOURNAL OF VIROLOGY, July 1972, p. 115-123

Vol. 10, No. 1
Printed in U.S.A.

Replicative Intermediates of Bacteriophage T7 Deoxyribonucleic Acid

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Received for publication 7 April 1972

After infection with bacteriophage T7, parental and newly synthesized deoxyribonucleic acid (DNA) exhibit an extremely fast sedimentation rate in neutral sucrose gradients. This fast-sedimenting component (intermediate I) has a sedimentation constant of about 1,500*S* and contains T7 DNA as determined by DNA-DNA hybridization experiments. Pulse-chase experiments indicate that the fast-sedimenting material is metabolically active and serves as a precursor to the formation of T7 DNA. Intermediate I contains about 2.5 to 7% of the total ^{3}H-labeled protein formed between 3 and 9.5 min after T7 infection. Treatment of intermediate I with Pronase results in the release of the DNA from the complex. At early times after infection, a second intermediate (intermediate II) can be detected which contains both parental and newly synthesized DNA sedimenting slower than intermediate I but 2 to 3 times as fast as mature T7 DNA. Intermediates I and II containing parental DNA are formed after infection of the nonpermissive host with an amber mutant in gene 1, a gene whose expression is necessary for the synthesis of most T7 proteins. The two intermediates are also observed when infection with T7 wild type is carried out in the presence of chloramphenicol.

Bacteriophage T7 offers several attractive features for studying the molecular events occurring during deoxyribonucleic acid (DNA) replication. The DNA of T7 is a relatively small molecule having a molecular weight of 26×10^6 daltons (26). After infection, 25 to 30, phage-specific proteins are made, and these account for the total coding capacity of T7 DNA (30). The genetics of T7 have been analyzed, and 19 genes have been identified and mapped (17, 27, 29). Six genes are necessary for phage DNA synthesis (27), and the proteins specified by four of these genes have been identified. Gene 1 codes for a phage-specific ribonucleic acid (RNA) polymerase (11). In the absence of this gene product, only the five earliest T7 proteins are made (30). One of these early proteins is a T7-induced polynucleotide ligase (3, 21). Genes 3 and 6 specify an endonuclease (8, 10, 23) and exonuclease (P. D. Sadowski, *in press*), respectively, both of which participate in the degradation of host DNA (10, 24). A second T7 endonuclease has recently been isolated, but its in vivo function is not known (7). Gene 5 specifies a T7 DNA polymerase (15).

Previous studies on DNA replication in T7-infected cells have demonstrated the presence of two distinct replicative intermediates, both of which have a sedimentation rate different from mature T7 DNA. Kelly and Thomas (19) and Carlson (6) have detected DNA which sediments about 1.6 times as fast as mature DNA. Electron microscope examination of this material indicates that it is a linear concatemer three to four times the length of T7 DNA (19). Masamune et al. (21) have observed that pulse-labeled DNA sediments in alkaline sucrose gradients as small 11*S* fragments. Similar results have been obtained by Hausman and LaRue (18) who examined the sedimentation profile of newly synthesized DNA occurring after infection of the nonpermissive host with several T7 amber mutants.

This report describes an additional replicative intermediate which exhibits an extremely fast sedimentation rate and may represent T7 DNA which is attached to a host component (possibly the bacterial membrane).

MATERIALS AND METHODS

Bacterial strains. *Escherichia coli* B, a nonpermissive host for amber mutants, was used as host for T7 phage infection. *E. coli* 011'(ATCC 27214) (27) was the permissive host for amber mutants.

Bacteriophage. Bacteriophage T7 was obtained from W. F. Studier.

Enzymes. T7 endonuclease I was the hydroxylapatite fraction prepared and assayed as described previously (7, 8).

Other materials. ^{3}H-Thymidine (15.6 Ci/mmole),

^{14}C-thymidine (35 mCi/mmole), ^{3}H-reconstituted protein hydrolysate, and carrier-free ^{32}P were purchased from Schwarz. Triton X-100 and Pronase were obtained from Sigma. The Pronase was digested for 2 hr at 37 C before use. Ribonuclease, obtained from Gallard-Schlesinger was heated for 5 min at 100 C before use.

Preparation of phage and phage DNA. Preparation of wild-type and amber mutants of T7 was carried out as described by Studier (27). The procedures used in the preparation of ^{3}H- and ^{32}P-labeled phage DNA and extraction of the DNA with phenol have been previously described (8).

DNA-DNA hybridization. DNA hybridization was performed by the method of Denhardt (12) as modified by Kelly and Thomas (19).

Labeling intracellular phage DNA and cell lysis. Unless indicated otherwise, *E. coli* B was grown at 30 C in M9 medium (2) supplemented with 0.2% Casamino Acids to a density of 5×10^{8} cells/ml. Phage T7 was added at a multiplicity of 5.0, and ^{3}H-thymidine was added to a final concentration of 10 μCi/ml. At various times after infection, 2.0-ml fractions were pipetted into 3 volumes of an ice-cold solution of 0.15 M NaCl-0.015 M ethylenediaminetetraacetic acid (EDTA), *p*H 8.0. The solution was centrifuged at 7,000 rev/min for 3 min in a Sorvall SS-34 rotor, and the supernatant fluid was discarded. The cells were lysed by the method of Knippers and Sinsheimer (20), except that the Brij 58 was replaced by Triton X-100. Approximately 1×10^{9} cells were suspended in 0.3 ml of 20% sucrose solution prepared in 0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (*p*H 8.0). To this suspension were added 0.1 ml of lysozyme (2 mg/ml in 0.25 M Tris, *p*H 8.0) and 0.1 ml of 0.01 M EDTA (*p*H 8.0). After incubating for 20 min at 0 C, 0.3 ml of a 5% Triton X-100 solution was added, and the suspension was held on ice for 20 min. The lysed cells were diluted to 1.5 ml with 0.05 M Tris-hyrochloride, *p*H 8.0. For some experiments, cells were lysed by the method of Frankel (13). The pellet obtained from infected cells, as described above, was suspended in 3.0 ml of a solution containing 0.01 M Tris-hydrochloride (*p*H 8.0)-0.05 M EDTA-0.05 M sodium azide and 2 mg of lysozyme per ml. The cells were incubated for 20 min at 0 C, and sarcosyl was added to a final concentration of 2%. Complete lysis was observed after 10 min at 0 C. This procedure does not disrupt phage particles. When disruption of the phage particles was desired, portions of the sarcosyl lysates were heated at 65 C for 20 min.

Labeling protein and DNA after T7 infection. *E. coli* B was grown in M9 medium (2) containing 2.5×10^{-3} mM $FeCl_3$ and Casamino Acids at a final concentration of 0.2 mg/ml. The cells were grown at 30 C to a density of 5×10^{8} cells/ml, and T7 phage was added at a multiplicity of 5.0. At 3 min after infection, protein was labeled by the addition of ^{3}H-protein hydrolysate to 4.0 μCi/ml. At 5.5 min after infection, DNA was labeled by the addition of ^{14}C-thymidine to 4.0 μCi/ml. Samples (2 ml) of the culture were taken at various times after infection and processed and lysed as described above.

Zone sedimentation through sucrose density gradients. (i) **Neutral sucrose gradients.** Portions of the cell lysate were centrifuged through 3.5 ml of a linear 5 to 20% sucrose gradient prepared in 0.05 M Tris-hydrochloride (*p*H 7.6)-0.05 M NaCl-0.001 M EDTA. The gradients were layered over a 1.0-ml shelf of 20% sucrose containing CsCl (1.33 g/ml). When the intracellular DNA was analyzed soon after infection, prior to the complete breakdown of host DNA, the lysates were vortexed for 20 sec before centrifugation was carried out. Unless indicated otherwise, 0.1 to 0.2 ml of the cell lysates was centrifuged for 50 min at 30,000 rev/min and 5 C in a Spinco SW50.1 rotor. Three-drop fractions were collected from the bottom of the tube, and the DNA was precipitated in the presence of 25 μg of salmon sperm DNA and 4.5% trichloroacetic acid. The solutions were passed over glass-fiber filters and washed with 0.01 N HCl. After the filters were dried, the radioactivity was determined.

(ii) Alkaline sucrose gradients. Prior to centrifugation, the cell lysates were incubated in 0.15 N NaOH for 20 min at room temperature. The denatured DNA was centrifuged through 4.5 ml of a linear 5 to 20% sucrose gradient prepared in 0.3 M NaOH-0.7 M NaCl-0.01 M Tris-hydrochloride-0.001 M EDTA. Centrifugation was for 90 min at 40,000 rev/min and 5 C in a Spinco SW50.1 rotor. Fractions were collected from the bottom of the tube, and the DNA was precipitated and processed as described above. In all experiments described, recovery of radioactivity from both neutral and alkaline sucrose gradient was at least 80%.

RESULTS

Analysis of intracellular parental DNA. T7 phage in which the DNA was labeled with ^{32}P was used to infect *E. coli* B, and at various times after infection the cells were collected and lysed by the lysozyme-Triton method. The DNA was analyzed after sedimentation in 5 to 20% sucrose gradients over a CsCl shelf. Just prior to centrifugation, the lysates were mixed with reference ^{3}H-T7 DNA. As shown in Fig. 1, two well resolved sedimenting species of parental DNA are observed. One component exhibits an extremely fast sedimentation rate and is recovered on the CsCl shelf. A second component which is detected 3 min after infection sediments at about twice the rate of mature T7 DNA. The amount of parental DNA sedimenting to the CsCl shelf 3 and 7 min after infection represents 22 and 35%, respectively, of the total radioactivity recovered from the gradient. It does not appear that the DNA recovered on the CsCl shelf is due to its being trapped in the lysate since, if reference T7 DNA is added during or after the lysis procedure and centrifuged in the usual manner, less than 1% of the DNA sediments to the CsCl shelf.

Analysis of parental and newly synthesized T7 DNA. The metabolic fate of parental and continuously labeled, newly synthesized DNA ex-

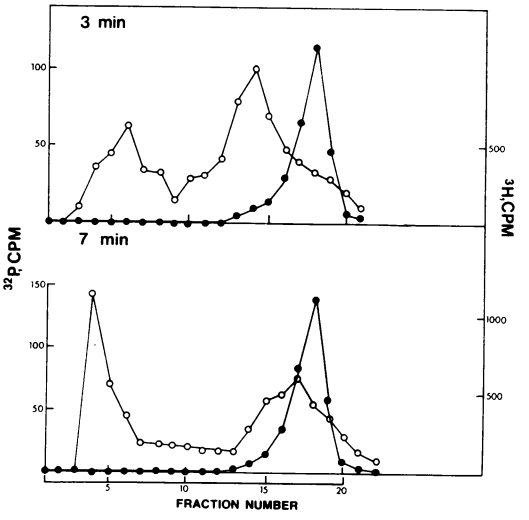

FIG. 1. *The fate of parental phage DNA after infection. E. coli B was infected with ^{32}P-labeled T7 wild-type phage as described in Materials and Methods except that the cells were grown at 32 C. At the indicated times after infection, samples were taken and the cells were lysed by the lysozyme-Triton method. Portions of the lysate were mixed with ^{3}H-labeled T7 DNA and centrifuged in neutral sucrose gradients containing a CsCl shelf. Centrifugation was for 50 min at 30,000 rev/min and 5 C in a Spinco SW50.1 rotor. Symbols: ○, ^{32}P-labeled DNA; ●, ^{3}H-labeled T7 marker DNA.*

amined at various times after infection by sedimentation analysis in neutral sucrose gradients is shown in Fig. 2. The pattern of label in the gradient demonstrates three distinct sedimenting species. Intermediate I consists of DNA sedimenting to the CsCl shelf. A second component, intermediate II, includes the DNA sedimenting at about 2 to 3 times as fast as T7 DNA. Finally, DNA is observed which has a sedimentation rate characteristic of mature T7 DNA. Both parental and newly synthesized DNA appear in each intermediate at about the same time after infection. Under the conditions of this experiment, the eclipse period has ended by 12 min after infection, and by 16 min about 10 infectious phage particles per cell have formed. The distribution of infectious phage particles after sucrose gradient centrifugation of the lysate prepared at 16 min after infection is shown in Fig. 2d.

Pulse-chase experiments. (i) Neutral sucrose sedimentation analysis. *E. coli* B was infected with ^{32}P-labeled T7 phage, and 6 min after infection ^{3}H-thymidine was added. Eight minutes after infection, the cells were centrifuged and suspended in medium containing unlabeled thymidine. A sample of the culture was taken at this time and at various times after infection, and the DNA from lysozyme-Triton cell lysates was examined after sedimentation in neutral sucrose gradients (Fig. 3). Immediately after the pulse, a major portion of the parental and newly synthesized DNA sediments to the CsCl shelf (Fig. 3a). When DNA synthesis is allowed to continue for 3 min in the presence of unlabeled thymidine, most of the ^{3}H-labeled DNA appears in the

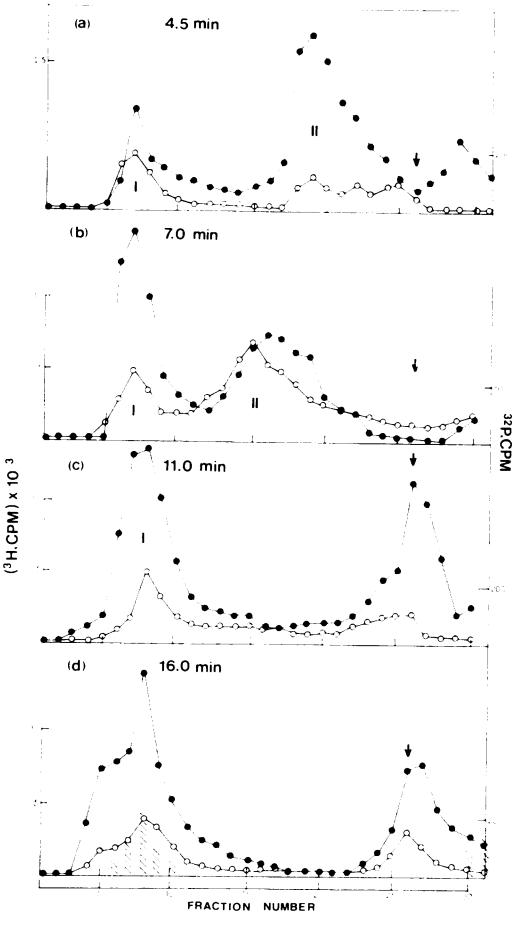

FIG. 2. *The fate of parental and newly synthesized DNA after infection. E. coli B was infected with ^{32}P-labeled T7 phage as described in Materials and Methods. ^{3}H-thymidine (10 μCi/ml) was added 4 min after infection. At the indicated times, samples were taken and the cells were lysed by the lysozyme-Triton method. Portions of the lysate were centrifuged as described in the legend of Fig. 1. Symbols: ○, ^{32}P-labeled parental DNA; ●, ^{3}H-labeled newly synthesized DNA. The arrow indicates the approximate position to which marker T7 DNA would be expected to sediment under these conditions. The cross-hatched areas (panel d) represent the amount of plaque-forming phage particles in each fraction relative to the amount contained in fraction 8. This fraction contained 7.5 × 10^{7} plaque-forming units/ml.*

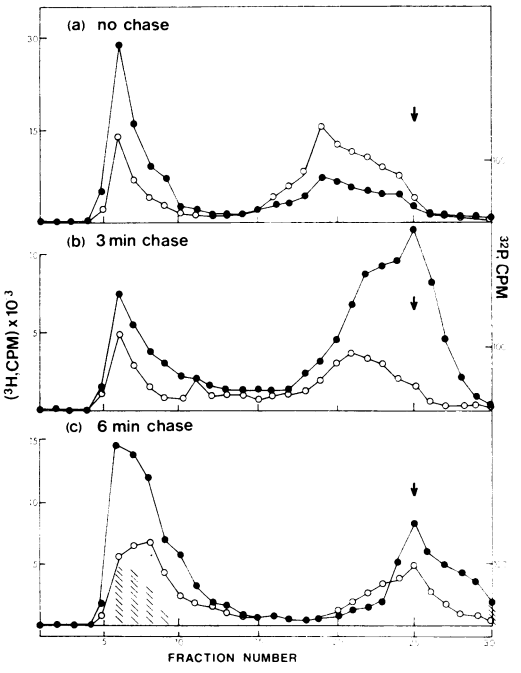

FIG. 3. *Pulse and chase experiments: neutral sucrose gradient analysis of parental and pulse-labeled DNA. E. coli B was infected with ^{32}P-labeled T7 phage at a multiplicity of 5 plaque-forming units per bacterium. ^{3}H-Thymidine was added 6 min after infection. At 8 min after infection, the cells were centrifuged and suspended in M9 medium containing 0.2% Casamino Acids and unlabeled thymidine at a final concentration of 1.5 mg/ml. During this procedure, there was no additional uptake of ^{3}H-thymidine. Samples of the culture were withdrawn at 8(a), 11(b), and 14(c) min after infection, pipetted into three volumes of 0.15 M NaCl-0.015 M EDTA (pH 8.0), and centrifuged. The cell pellets were lysed by the lysozyme-Triton method. Portions of the cell lysates were centrifuged in neutral sucrose gradients as described in the legend to Fig. 1. Symbols: ○, ^{32}P-labeled parental DNA; ●, ^{3}H-pulse-labeled DNA. The arrow indicates the approximate position to which marker T7 DNA would be expected to sediment under these conditions. The cross-hatched areas (panel c) represent amount of the plaque-forming particles in each fraction relative to the amount contained in fraction 6. This fraction contained 8 × 10^{6} plaque-forming units/ml.*

gradient at a position expected for mature T7 DNA and as a fraction sedimenting 1.4 times the rate of T7 DNA. However, after a 6-min chase, most of the ^{3}H-labeled DNA has resumed a fast sedimentation rate and can be recovered on the CsCl shelf. This is not due to encapsulation of the DNA in mature phage since those fractions of the sucrose gradient containing the fast-sedimenting DNA have extremely low levels of infectious particles (Fig. 3c). A possible explanation for this observation is presented below. The fate of parental DNA during the pulse-chase experiment is also shown in Fig. 3. Immediately after the pulse, a portion of parental DNA sediments as intermediate I, while a second fraction sediments about twice as fast as mature T7 DNA. The amount of parental DNA sedimenting to the CsCl shelf remains constant during the chase periods. However, after the 6-min chase, DNA sedimenting with mature T7 DNA is observed (Fig. 3c).

Since the above experiments did not demonstrate a complete utilization of the ^{3}H-labeled DNA in intermediate I for the formation of mature T7 DNA, pulse-chase experiments were carried out in which newly synthesized DNA was analyzed in lysates prepared by the high-temperature lysozyme-sarcosyl method. Treatment of lysates under these conditions disrupts phage particles. The results of these experiments demonstrated that essentially all of the ^{3}H-labeled DNA was present as intermediate I immediately after the pulse was converted to a form with a sedimentation rate identical to mature T7 DNA.

(ii) Alkaline sucrose sedimentation analysis. The sedimentation profile for newly synthesized DNA in alkaline sucrose gradients is shown in Fig. 4. The protocol for the experiment was as described above except that the infecting phage DNA was not labeled. The sedimentation pattern for the DNA in neutral sucrose gradients examined at 8, 11, and 14 min after infection was essentially the same as that shown in Fig. 3. Immediately after the pulse, the DNA in alkaline sucrose appears quite heterogeneous and sediments with marker DNA and from 1.1 to 1.6 times as fast as T7 DNA (Fig. 4a). After a 3-min chase, fragments sedimenting slower than T7 DNA are also observed (Fig. 4b). When DNA synthesis is allowed to continue for an additional 7 min in the presence of unlabeled thymidine, most of the ^{3}H-labeled DNA sediments with the reference marker (Fig. 4d). Based on the previous findings of Kelly and Thomas (19), the DNA sedimenting faster than T7 DNA in alkaline sucrose is a concatemer consisting of linear single strands of repeating genomes covalently linked together. The largest DNA molecules observed in Fig. 3a would contain about three T7 DNA genome equivalents.

Infection with a T7 mutant defective in head assembly. The results of the experiment described in Fig. 3 showed that the newly synthesized DNA in intermediate I could be chased into a slower sedimenting form, but at later times after infection this material resumed a fast sedimentation rate and could be recovered on the CsCl shelf (Fig. 3c). Possibly the DNA resumed a fast

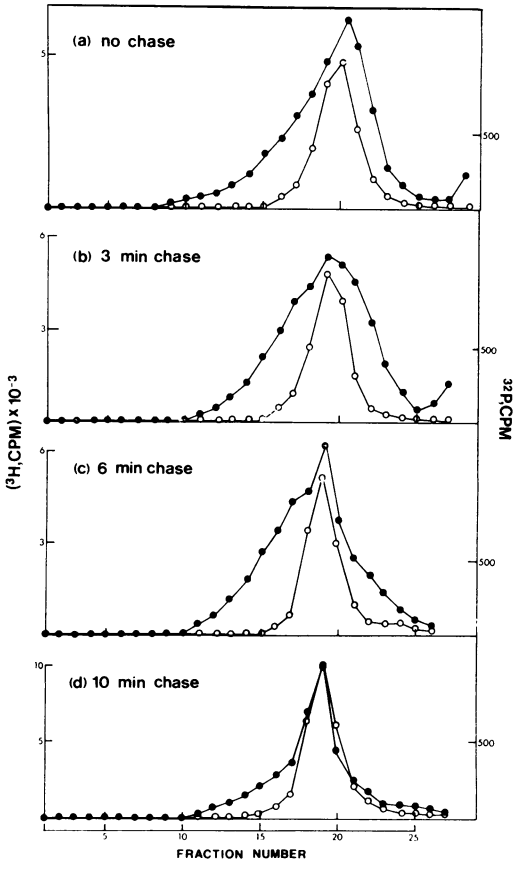

FIG. 4. *Pulse and chase experiment: alkaline sucrose gradient analysis of pulse-labeled DNA. The details of the experiment were identical to those described in the legend to Fig. 3, except that the parental DNA was not labeled. Samples of the culture were withdrawn at 8(a), 11(b), 14(c), and 18(d) min after infection, and the cell pellets were lysed by the lysozyme-Triton method. Portions of the lysate were centrifuged in alkaline sucrose gradients as described in Materials and Methods. Symbols: ○, ^{32}P-labeled T7 marker DNA; ●, ^{3}H-pulse-labeled DNA.*

sedimentation rate due to its being packaged in the phage head or alternatively the DNA becomes associated with some cellular component where final maturation and packaging takes place. To investigate this point further, newly synthesized DNA was analyzed after infection of the nonpermissive host with an amber mutant in gene 9. Mutants in this gene synthesize DNA at a normal rate (27) but are defective in the formation of complete head structures (28). The gene 9 protein appears to be involved in the condensation of the capsid protein (gene 10 product) to produce the head structure (28).

The results of a pulse-chase experiment in which the newly synthesized DNA was analyzed in neutral sucrose gradients is shown in Fig. 5. The sedimentation profile of the DNA is similar to that observed after wild-type infection. A considerable portion of the DNA sedimenting as intermediate I can be chased into slower sedimenting material (Fig. 5b). However at later times after infection, this DNA has resumed a fast sedimentation rate and is recovered on the CsCl shelf (Fig. 5c–d). This material recovered on the shelf has a sedimentation constant of at least 1,000*S* when compared with bacteriophage T4 (850*S*). The newly synthesized DNA formed after infection with gene 9 mutants has also been analyzed in alkaline sucrose gradients (Fig. 6). It appears that the DNA is metabolized in two distinct stages. First there is the formation of fast-sedimenting material (Fig. 6a) which is subsequently converted to a component sedimenting close to T7 DNA (Fig. 6b). However, in the absence of the gene 9 product, fast-sedimenting

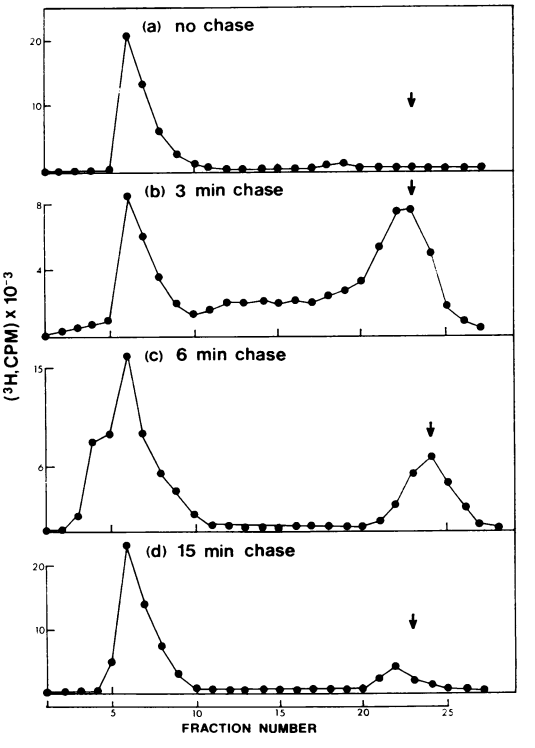

FIG. 5. *Pulse and chase experiment; neutral sucrose gradient analysis of pulse-labeled DNA after T7 am17 infection. E. coli B (nonpermissive host) was infected with T7 am17 amber mutant in gene 9, at a multiplicity of 5.0. The details of the experiment were identical to those described in the legend to Fig. 3. Symbols: ●, ^{3}H-pulse-labeled DNA. The arrow indicates the position to which marker ^{32}P-labeled T7 DNA sediments.*

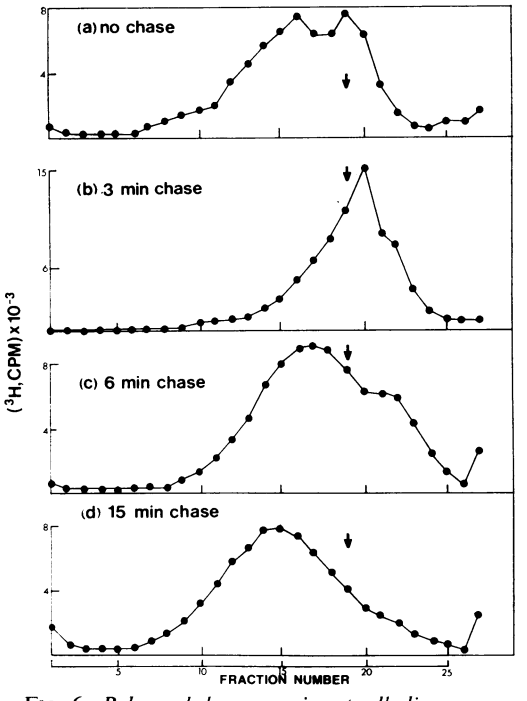

FIG. 6. *Pulse and chase experiment: alkaline sucrose gradient analysis of pulse-labeled DNA after T7 am17 infection. Portions of the lysates, prepared as described in the legend to Fig. 5, were centrifuged in alkaline sucrose gradients as described in Materials and Methods. Symbols ●, ^{3}H-pulse-labeled DNA. The arrow indicates the position to which marker ^{32}P-labeled T7 DNA sediments.*

DNA accumulates and is not metabolized further (Fig. 6c–d). The fast-sedimenting material observed at later times after infection is presumably a T7 concatemer, and the DNA present after a 15-min chase (Fig. 6d) would consist of about five T7 genome equivalents.

Properties of intermediate I. Newly synthesized DNA sedimenting as intermediate I is observed when cell lysates are prepared by the lysozyme-Triton or sarcosyl method. The intermediate I DNA in both types of lysates has a sedimentation coefficient of at least 1,500*S* when compared with bacteriophage T4 (850*S*) and appears to be predominantly T7 DNA as determined by hybridization experiments (Table 1).

Evidence for a T7 DNA-protein complex. Several lines of evidence indicate that a major portion of the newly synthesized DNA is present as a DNA-protein complex. In these experiments, protein and newly synthesized DNA were labeled with ^{3}H and ^{14}C, respectively, as described above. The infected cells were collected at 9.5 min after infection and lysed by the lysozyme-Triton or sarcosyl method. Portions of the lysate were

TABLE 1. *DNA-DNA hybridization experiment*

Test DNA	Acid-insoluble counts pre min per filter	Radioactivity bound to filters (counts/min)[a]	
		E. coli DNA[b]	T7 DNA[b]
T7	920	9	480
Intermediate I[c] (isolated)	1,660	42	692
E. coli DNA	4,340	498	31

[a] Blank values (<1% of input radioactivity) are subtracted.

[b] Filters contained 8.5 μg of T7 DNA, or 16 μg of *E. coli* DNA.

[c] Intermediate I containing ^{3}H-labeled DNA was prepared as described in the legend to Fig. 8.

centrifuged in neutral sucrose gradients containing a CsCl shelf. When lysates are prepared by the lysozyme-sarcosyl method, about 7% of the total ^{3}H-labeled protein sediments to the CsCl shelf along with the ^{14}C-labeled DNA (Fig. 7a). When the labeled protein and DNA sedimenting to the shelf are isolated, dialyzed, and resedimented under the same conditions, most of this material again exhibits a fast sedimentation rate (Fig. 7b). Similar results have been obtained when lysates are prepared by the lysozyme-Triton method, although under these conditions 2.5% of the ^{3}H-protein cosediments with the ^{14}C-DNA to the CsCl shelf. It has been found that when the material sedimenting to a CsCl or 70% sucrose shelf is isolated and dialyzed, most of the DNA and protein does not resediment to the CsCl shelf, presumably due to a fragmentation of the complex.

Experiments have also been carried out in which intermediate I, containing ^{3}H-labeled, newly synthesized DNA, was isolated from sarcosyl lysates and treated with various enzymes. As shown in Fig. 8a, Pronase treatment converts 60% of the DNA in intermediate I to a form which sediments with mature T7 DNA. Incubation with ribonuclease has no effect on the sedimentation rate of this material. T7 endonuclease I (8, 10, 23) is capable of degrading the DNA in intermediate I, and, under the assay conditions used, the degradation product sediments at a rate slower than mature T7 DNA (Fig. 8b). The fragmentation of the DNA observed after sedimentation in neutral sucrose is identical to previous results obtained when the enzyme is incubated with purified T7 DNA (9).

Effect of chloramphenicol and infection with an amber mutant in gene 1 on the formation of intermediate I. The protein specified by gene 1 of T7

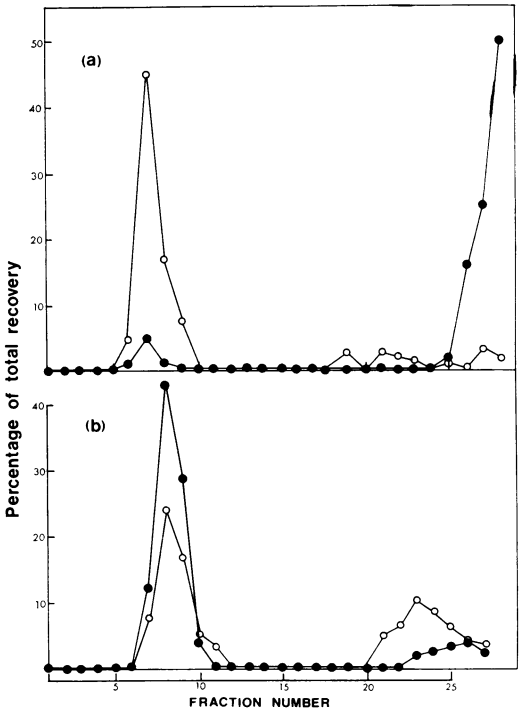

FIG. 7. *Association of protein and newly synthesized T7 DNA. E. coli B was infected with T7 wild-type phage at a multiplicity of 5.0. Labeling of protein and DNA with ^{3}H and ^{14}C, respectively, was performed as described in Materials and Methods. Samples of the culture were withdrawn at 9.5 min after infection, and the cells were lysed by the lysozyme-sarcosyl method. Portions of the lysates were centrifuged in neutral sucrose gradients containing a CsCl shelf, for 45 min at 30,000 rev/min and 5 C in a Spinco SW50.1 rotor. A sample of each fraction was assayed for acid-insoluble radioactivity (panel a). The remainder of the fractions containing ^{3}H-labeled protein and ^{14}C-labeled DNA which had sedimented to the CsCl shelf were pooled and dialyzed for 90 min at 4 C against 0.05 M Tris-hydrochloride (pH 7.6)-0.05 M NaCl-0.001 M EDTA. The dialyzed material was resedimented as described above, except that the centrifugation time was 50 min. The entire volume from each fraction was assayed for acid-insoluble radioactivity (panel b). Symbols: ○, ^{14}C-labeled newly synthesized DNA; ●, ^{3}H-labeled protein.*

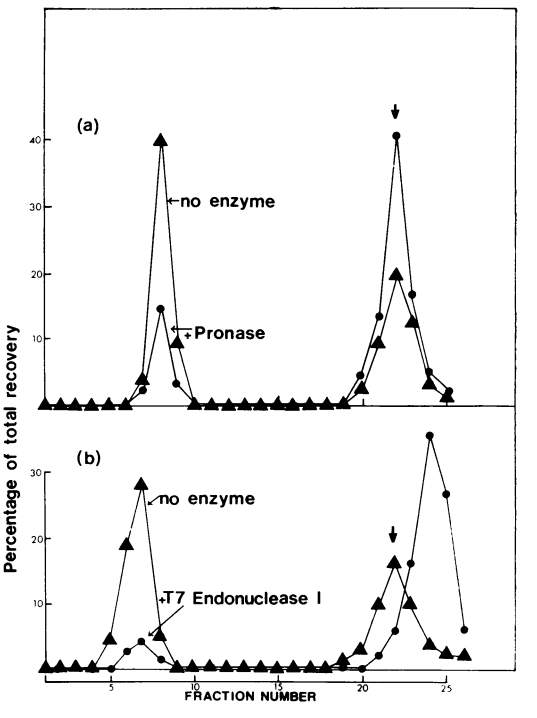

FIG. 8. *The effect of Pronase and T7 endonuclease on the sedimentation rate of intermediate I. E. coli B was infected with T7 phage at a multiplicity of 5.0. ^{3}H-thymidine (10 μCi/ml) was added 6 min after infection, and after an additional 4-min incubation period the cells were collected and lysed by the lysozyme-sarcosyl technique as described in Materials and Methods. Portions of the lysate were centrifuged in neutral sucrose gradients containing a CsCl shelf, for 45 min at 30,000 rev/min and 5 C in a Spinco SW50.1 rotor. The ^{3}H-labeled DNA sedimenting to the shelf was collected and dialyzed as described in the legend to Fig. 7. A portion of this DNA (0.15 ml) was incubated with 125 μg of Pronase for 2 hr at 37 C (panel a). Another portion of the DNA (0.2 ml) was incubated with T7 endonuclease I in the standard reaction mixture at pH 8.0 (8). Incubation was for 30 min at 34 C in the presence of 20 units of enzyme (panel b). The reaction was stopped by the addition of 2 μmoles of EDTA. As a control for each experiment, the DNA was incubated under identical conditions in the absence of enzyme. After enzyme treatment, the solutions were mixed with ^{32}P-labeled T7 DNA and resedimented as described above. Each collected fraction was assayed for acid-insoluble radioactivity. The distribution of radioactivity for the control and enzyme-treated DNA are plotted together. The arrow indicates the position to which marker ^{32}P-labeled T7 DNA sediments.*

is a phage-induced RNA polymerase (11). In the absence of a functional T7 RNA polymerase, only the five earliest T7 genes are expressed (30). As shown in Table 2, ^{32}P-labeled parental DNA can form intermediate I if wild-type infection is carried out in the presence of chloramphenicol or if the infecting phage DNA has a mutation in gene 1. It has also been found that DNA sedimenting as intermediate II is formed under these conditions. These results would therefore suggest that the formation of intermediate I or II does not require a specific phage function.

DISCUSSION

During the replication of T7 DNA, several intermediates with distinct sedimentation properties can be detected. Two major components containing both parental and newly synthesized DNA are observed during the eclipse period.

TABLE 2. *Effect of chloramphenicol and infection with T7 am193 on the formation of intermediate I*[a]

Conditions of infection	Intermediate I percentage of total radioactivity				
	7[b]	9	11	15	25
T7 Wild type	13	36	30		
T7 Wild type + chloramphenicol	28	31	33	28	29
T7 *am*193	19	19	14		23

[a] *E. coli* B (nonpermissive host) was infected at a multiplicity of 5.0 with ^{32}P-labeled T7 wild-type of T7 *am*193 (amber mutant in gene 1). Chloramphenicol when used during wild-type infection was added just prior to phage infection at a concentration of 100 μg/ml. At various times after infection, samples were taken, and the cells were lysed by the lysozyme-Triton method. Portions of the lysate were centrifuged as described in the legend to Fig. 1. The amount of intermediate I is taken as the percentage of total radioactivity recovered on the CsCl shelf.

[b] Minutes after infection.

Intermediate II, which is present at early times after infection, includes those DNA species sedimenting 2 to 3 times as fast as T7 DNA. The nature of this material and its role in T7 DNA synthesis is not known at the present time. The second intermediate sediments as a 1,500*S* component and appears to exist as a DNA-protein complex. The results of pulse-chase experiments strongly suggest that the newly synthesized DNA present in the complex (intermediate I) is a precursor to the formation of mature T7 DNA. These data taken together suggest that DNA synthesis occurs in association with some cellular component, possibly the bacterial membrane. The general implication that the bacterial membrane may have an important role in DNA synthesis has been previously described for several other phage systems including φX174 (20), φR (5), T4 (1, 22), P22 (4), and λ (16, 25). It appears that in the T7 system parental DNA can enter the complex in the absence of phage-directed protein synthesis. Phage T4 (22) and λ (16) on the other hand seem to require a phage-induced function before parental DNA becomes associated with the complex. The association of pulse-labeled DNA with the complex appears to be transient, and by 11 min after infection the DNA is released and sediments as 32*S* material. At later times after infection, but before appreciable amounts of progeny virus particles have formed, the 32*S* DNA resumes a fast sedimentation rate in neutral sucrose gradients. Similar results are obtained when infection is carried out with a T7 mutant defective in the formation of complete head structures. These results suggest a scheme of events where the pulse-labeled DNA is released from the complex but reassociates with some cellular component which is the site of DNA maturation (formation of T7 length molecules) and phage assembly. The results of these studies also suggest that in the absence of the formation of a complete head structure, as occurs after infection with a gene 9 mutant, T7 DNA maturation does not occur. A similar situation also seems to exist for bacteriophage T4 (14). We have also found that T7 DNA maturation does not occur after infection of the nonpermissive host with an amber mutant in gene 19. The protein specified by this gene is not a part of the phage capsid nor is it necessary for capsid assembly (28). The function of gene 19 is not known, but it may be directly involved in the maturation process. Similar conclusions concerning the role of genes 9 and 19 in the maturation process have been previously obtained by Hausmann and LaRue (18) and Studier (28).

ACKNOWLEDGMENT

This investigation was supported by Public Health Service research grant AI-10238 from the National Institute of Allergy and Infectious Diseases.

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