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Role of Gene 52 in Bacteriophage T4 DNA Synthesis

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In an attempt to elucidate the mechanism of delayed DNA synthesis in phage T4, *Escherichia coli* B cells were infected with H17 (an amber mutant defective in gene 52 possessing a "DNA-delay" phenotype). The fate of ^{14}C-labeled H17 parental DNA after infection was followed: we could show that this DNA sediments more slowly in neutral sucrose than wild-type DNA 3 min postinfection. In pulse-chase experiments progeny DNA was found to undergo detachment from the membrane at 12 min postinfection. Reattachment to the membrane was found to be related to an increase in rate of DNA synthesis. A nucleolytic activity that is absent from cells infected by wild-type phage and from uninfected cells could be detected in extracts prepared from mutant-infected cells. In contrast, degradation of host DNA was found to be less extensive in *am* H17 compared with wild-type infected cells. Addition of chloramphenicol to mutant-infected cells 10 min postinfection inhibited the appearance of a nuclease activity on one hand and suppressed the "DNA-delay" phenotype on the other hand. We conclude that the gene 52 product controls the activity of a nuclease in infected cells whose main function may be specific strand nicking in association with DNA replication. This gene product might directly attack both *E. coli* and phage T4 DNA, or indirectly determine their sensitivity to degradation by another nuclease.

Initiation of T4 DNA replication must be a complex process from a biochemical point of view as asserted by the multiplicity of genes that appear to be involved (7). A variety of structural models for T4 replication have been proposed, including the participation of circular (8, 11, 35) and more complex recombination derived intermediates (25). Initiation of DNA replication has been suggested to start at a single, specific site on the genome (22) to be used repeatedly (35). In contrast, Delius et al. (4) have observed replicative loops (interpreted as initiating points) scattered along the parental genome. Studies on conditional lethal mutants blocked in the initial replication process should provide insight into the nature of T4 replication intermediates. We have recently shown (26) that cells infected with a "DNA-delay" mutant in gene 52 show defective concatemer formation and have implicated the regulation of some nuclease activity in this effect. The present study was undertaken to elucidate some of the processes occurring during the initiation of DNA synthesis in phage T4. We have sought answers to these two questions: (i) How is the gene 52 product involved in the early process of replication? (ii) Does the gene 52 product directly, or indirectly, affect some nuclease activity?

These results are part of a thesis submitted by Y. Naot in partial fulfillment of the requirements of the D.Sc. degree at the Department of Biology, Technion-Israel Institute of Technology, Haifa, Israel.

MATERIALS AND METHODS

Chemicals. Thymidine-*methyl*-^3H (14.3 Ci/mmol) and thymidine-2-^{14}C (50.8 mCi/mmol) were obtained from Schwarz BioResearch, Inc., Orangeburg, N.Y. Chloramphenicol was obtained from Abic, Ramat-Gan, Israel. 2-Deoxyadenosine was from Sigma Chemical Co. and Pronase grade B from Calbiochem. Sarkosyl NL30 was the gift of the Geigy Chemical Corp.

Bacterial strains. *Escherichia coli* strains B and CR63 were obtained from F. W. Stahl.

Bacteriophage strains. T4Dtd8Bx4 was obtained from I. Tessman. T4Dam$^+$ (wild type T4D) and amber mutant H17 (gene 52) were from R. S. Edgar through F. W. Stahl. *am* H17 was genetically purified by once back-crossing to wild type (at a multiplicity of infection [MOI] = 5 each).

Media. The Tris-glucose medium used was the same as described before (31).

Radioactive phage particles. Labeling of phage particles was done as described on an earlier occasion (29).

Thymidine-methyl-^{3}H incorporation. The assay for ^{3}H-thymidine incorporation as acid-insoluble material on a membrane filter (25 mm, 0.45 μm) was done as described previously (31).

Preparation of infected cells and cell lysates: Method 1. *E. coli* B cells were prelabeled with ^{3}H-glycerol as previously described (30) and infected with ^{14}C-labeled phage at a multiplicity of 5 to 10 phage particles per bacterium at 26 C in the presence of 20 μg of L-tryptophan per ml. After 2 min for adsorption, the infected bacteria were diluted in warm Tris-glucose medium supplemented with 0.1% nonradioactive glycerol and were aerated at 37 C (zero time). At 1 min after infection, phage development was arrested by pouring the infected culture onto an equal volume of cold 0.1 M KCN. After 10-fold concentration in Tris-glucose medium in $MgSO_4$ (2.5×10^{-3} M), an equal volume of an ice-cold lysing solution, specified before (30), was immediately added. After 10 min of incubation at 37 C, Sarkosyl was added to 0.1%. The lysates were allowed to clear during overnight storage under refrigeration (4 C).

Method 2. *E. coli* B cells were grown to a density of 2×10^8 cells/ml in Tris-glucose medium. The cells were infected at a density of 2×10^8 cells/ml with 5 to 10 phage particles per cell at 26 C. After 2 min the infected bacteria were diluted in warm Tris-glucose medium supplemented with deoxyadenosine (250 μg/ml) (T = 0) and aerated at 37 C. At 6 min postinfection 10 μCi of ^{3}H-thymidine per ml was added to the growth medium. In pulse-chase experiments, nonlabeled thymidine was added to a final concentration of 1.8 mg/ml (10,000-fold excess) at 7.9 min postinfection. Control experiments showed no further incorporation of ^{3}H. Samples were withdrawn at the time indicated and poured onto an equal volume of cold 0.1 M KCN. Lysis occurred as described above.

Method 3. *E. coli* B cells were grown and infected as described above. For lysis, the medium specified previously (30) was added. When DNA was sedimented through neutral sucrose gradients, lysis occurred with 0.4% sodium dodecyl sulfate (SDS), and the lysates were kept overnight in the cold. Before analysis, the lysates were diluted twofold and subjected to Pronase (5 mg/ml) digestion for 3 h at 37 C. Pronase was preincubated for 24 h at 37 C at a concentration of 10 mg/ml in 0.05 M Tris, 0.15 M NaCl, and 0.005 M EDTA, pH 7.2. When DNA was analyzed through alkaline sucrose gradients, Pronase digestion was omitted. Under these conditions (in the absence of Pronase digestion), no material which sediments faster than reference DNA in alkaline sucrose gradients can be detected at early times after infection.

Method 4. *E. coli* B cells were prelabeled with ^{3}H-thymidine as described by Kutter and Wiberg (18). After infection (MOI = 10), infected cells were diluted 10-fold in growth medium containing 180 μg of nonradioactive thymidine per ml and 250 μg of deoxyadenosine per ml and aerated at 37 C. Samples (1 ml, 2×10^8 bacteria/ml) were withdrawn at the time specified onto an equal volume of the lysing medium previously described: 200 μg of lysozyme per ml and 0.1 M EDTA, pH 8 (18). After 1 min of incubation at 65 C, SDS was added at a final concentration of 0.1%. Pronase (0.5 mg/ml, preheated 10 min at 85 C at a concentration of 10 mg/ml) was admixed with the detergent. The mixture was kept overnight in the cold. Before analysis in alkaline sucrose gradients, the mixture was incubated for 10 min at 65 C.

Zone sedimentation in sucrose gradients. Linear neutral or alkaline sucrose gradients were built as described before (30). ^{14}C-labeled phage T4Dam$^+$ served as 1,000*S* marker in neutral sucrose gradients. T4 marker DNA (1.3×10^8 daltons) was released from ^{14}C-labeled phage by 10 min of incubation at 65 C in the presence of 0.025 M EDTA, pH 8.5, and 0.6% Sarkosyl followed by Pronase (5 mg/ml) digestion for 3 h at 37 C as described for intracellular DNA. Marker DNA was spun in a different tube in every run. When bacterial DNA was analyzed in sucrose gradients, the gradients were built up from 10 to 40% sucrose (4.8 ml), made up in 0.3 N NaOH, 0.7 M NaCl, 0.001 M EDTA, pH 12. Denaturation of bacterial DNA was on top of the gradient in 0.3 N NaOH for 20 min at room temperature. Release of T4 DNA from mature ^{14}C-labeled phage was carried out on top of the gradient in 0.3 N NaOH for 15 min at 4 C before centrifugation. Centrifugation was carried out in a Spinco model L ultracentrifuge at 4 C in an SW25, SW39, or SW50 rotor. Fractions were collected and analyzed for acid-precipitable radioactivity as previously described (29). Molecular weights for native DNA in neutral gradients were calculated as described before (29). For the molecular weight of denatured DNA in alkaline gradients, the plot represented in Fig. 2, reference 20 was used.

Preparation of cell extracts for enzyme assays. Cells were grown at 37 C to 2×10^8 cells/ml in Tris-glucose medium, concentrated 10-fold, and infected with 5 to 10 phage particles per bacterium. After 2 min (26 C) for adsorption, the infected cells were diluted 10-fold into warm medium at 37 C (T = 0). The infected culture was aerated for 20 min. Growth was arrested by pouring onto an equal volume of chilled medium supplemented with a final concentration of 10 mM NaN_3. Infected bacteria were spun and resuspended in 1 ml of medium supplemented with a final concentration of 0.1 mM EDTA and 2 mM 2-mercaptoethanol. The cells were broken in an MSE sonifier by 1 min of sonic oscillation. The disrupted cell suspension was centrifuged at 14,000 rpm (Sorvall RC2-B) for 30 min to remove cell debris, and the deoxyribonuclease activity of the supernatant fluid was immediately assayed.

Assay of deoxyribonuclease. The assay mixture in a total volume of 0.6 ml consisted of 10 μmol of Tris-hydrochloride buffer (pH 8), 10 μmol of $MgSO_4$, 2 μmol of 2-mercaptoethanol, 0.1 μmol of EDTA, 200 nmol of ATP, 2 nmol (nucleotide equivalent) of ^{14}C-labeled T4 DNA (sheared 1 min in an MSE sonifier), and 250 μg (protein) of extract. Incubation was carried out at 37 C for 30 min. The reaction was terminated by adding 0.1 ml of carrier solution (6 mg of bovine serum albumin per ml) and 0.6 ml of 10% trichloroacetic acid. After 15 min at 0 C, followed by centrifugation, the entire supernatant fraction was

transferred to 10 ml of scintillation fluid consisting of 1,200 ml of Triton X-100 (Packard), 2 liters of toluene, and 140 ml of Liquifluor (New England Nuclear Corp.). Protein concentrations were determined by the method of Lowry et al. (21). The assay was linear with respect to protein concentration and time of incubation. Identical results were obtained when 15 nmol instead of 2 nmol (nucleotide equivalent) of ^{14}C-labeled DNA was added to the assay mixture, or when 45 nmol of *E. coli* tRNA was added.

RESULTS

Association of phage DNA with the membrane of E. coli B. Since it is well known that T4 DNA rapidly attaches to the bacteria cell membrane after infection (1, 5, 6, 23), it was of interest to test whether the DNA of a DNA-delay mutant in gene 52 which synthesizes DNA at an initial reduced rate (26, 40) still possesses the ability to associate with the host cell membrane. Results (Fig. 1) show that 40% of parental ^{14}C-labeled H17 and wild-type DNA cosediment with ^{3}H-labeled host membrane as soon as 1 min postinfection. (In the experiment with "wild-type" DNA, td8 [thymidine requiring] served as wild-type control since the uptake of ^{14}C-thymidine into this mutant was twice as good as into ordinary wild-type phage without the td mutation.) At 3 min postinfection, when more than 95% of the phage has adsorbed, 50% of the ^{14}C radioactivity cosediments with ^{3}H-labeled host cell membrane (data not shown). Hence, we conclude that there is no defect in

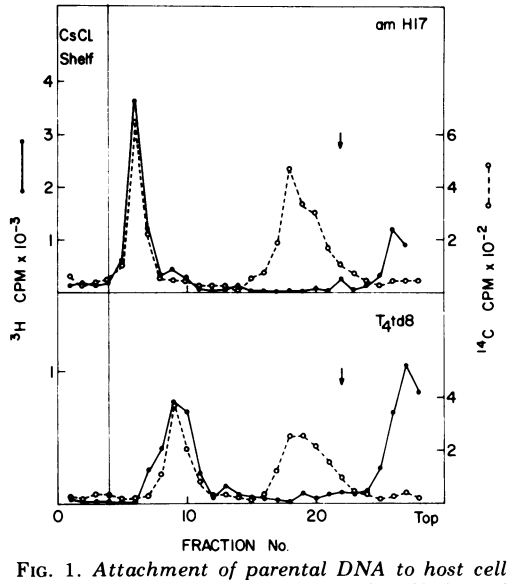

FIG. 1. *Attachment of parental DNA to host cell membrane. E. coli B cells prelabeled with ^{3}H-glycerol were infected with ^{14}C-labeled td8 or H17. Infected cells were lysed 1 min after infection as described in text (Method 1). Sedimentation in neutral sucrose gradients (5–30% [wt/vol] containing a 0.6-ml underlayer of 1.7 g of CsCl per cm^{3} and 40% [wt/vol] sucrose) was carried out in a Spinco model L-2 ultracentrifuge at 4 C for 50 min at 32,000 rpm (SW50 rotor). The position of mature phage DNA is indicated by the arrow. Input ^{3}H radioactivity: T4td8, 6,000 counts/min; am H17, 8,500 counts/min. Input ^{14}C radioactivity: T4td8, 2,774 counts/min; amH17, 2,932 counts/min. Recoveries were about 80 to 90% for ^{3}H and ^{14}C, respectively.*

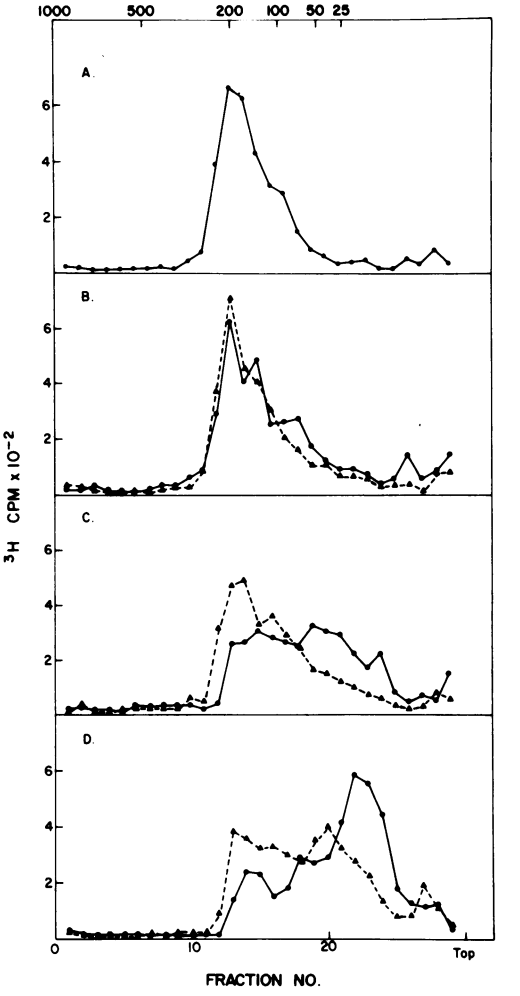

FIG. 2. *Host DNA degradation following phage infection. E. coli B cells were prelabeled with ^{3}H-thymidine, infected with phage, and lysed as described in text (Method 4). A, Noninfected bacteria; B, 3 min; C, 5 min; D, 7 min postinfection with am^{+} (○) or with H17 phage (▲). Sedimentation in alkaline sucrose gradients (10–40%) was carried out at 4 C, 40,000 rpm for 80 min in an SW50 rotor. The position obtained for reference ^{14}C-T4-DNA released from whole phage and denatured on top of the gradient was used to calculate the scale of approximate molecular weight (× 10^{6}) at the top of the graph. ^{3}H input was 2,500 to 3,200 counts/min. Recoveries of the order 93 to 100%.*

the association of *am* H17 parental phage DNA to host cell membrane. However, the H17-membrane complex sediments more rapidly(~230*S*) than the wild-type complex (~200*S*). These sedimentation coefficients are in accord with previous studies of early replication complexes containing host cell membrane (1, 23). Both bacterial and phage DNA molecules are associated with cell membrane during the initial stages of infection (5). Host cell DNA is subsequently detached by degradation (5, 18). It was therefore plausible to investigate whether the difference in sedimentation coefficient of H17 and wild-type membrane complex depicted in Fig. 1 might be due to defective degradation of host cell DNA in gene 52 mutant-infected cells.

Defective degradation of host cell DNA after mutant infection. Previous studies have shown that, after wild-type T4 phage infection, the bacterial DNA is first converted to a slower sedimenting, but still high-molecular-weight, form and then is subjected to extensive breakdown into acid-soluble material (3, 13, 18, 34). It was thus of interest to determine the sequence of *E. coli* DNA degradation following mutant infection. In the following studies, the size of prelabeled host cell DNA at early times after infection was determined by alkaline sucrose gradient centrifugation. Results (Fig. 2) show that after wild-type infection bacterial DNA is degraded as soon as 5 min after infection (Fig. 2C). In contrast, the bulk of the host DNA examined at the same time after mutant infection (Fig. 2C) was similar in size to that of uninfected cells (Fig. 2A). Furthermore, the difference in molecular size is increased at 7 min after infection (Fig. 2D). We thus conclude that, following gene 52 infection, degradation of host DNA is delayed. These results are in accord with the sensitivity of phage production to hydroxyurea in *E. coli* B cells infected with H17 (26).

Fate of parental phage DNA postinfection. The deoxyribonucleases induced by T4 phage infection (15, 36) might have a dual role in attacking both *E. coli* and phage T4 DNA (18, 27). In analogy with the gene 46 and 47 products, the gene 52 product might also directly attack both *E. coli* and T4 DNA or indirectly determine their sensitivity to degradation by another nuclease. Therefore we have analyzed the fate of parental phage DNA after infection with *am* H17 and wild-type phage. Neutral sucrose gradients were used to compare wild-type and H17 ^{14}C-labeled parental DNA molecules at 3 min after infection of ^{3}H-prelabeled *E. coli* B cells. It is seen in Fig. 3A that ^{14}C-labeled parental wild-type DNA is slightly faster sedimenting than mutant DNA. Al-

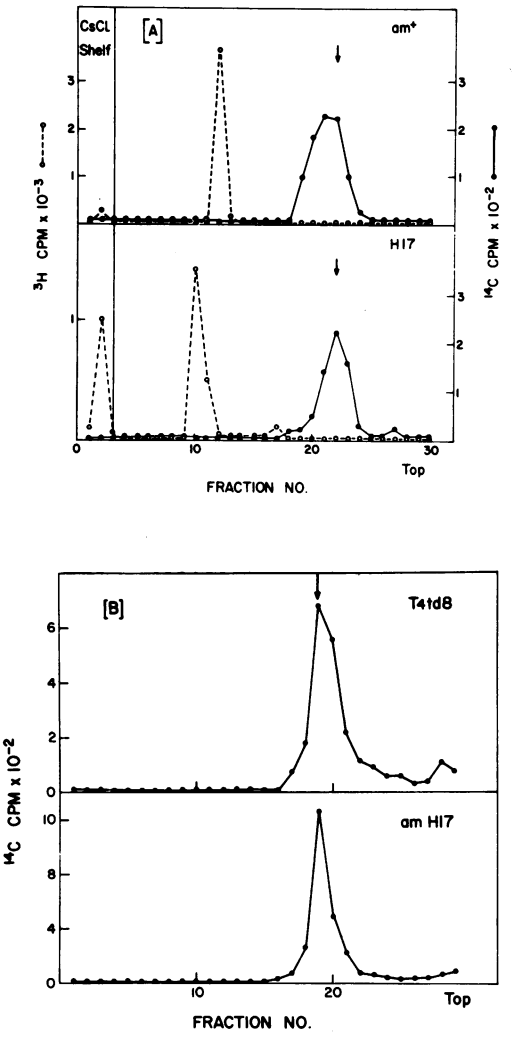

FIG. 3. *Sedimentation analysis of* ^{14}*C-labeled parental DNA 3 min postinfection. A, E. coli B cells were prelabeled with* ^{3}*H-thymidine (0.1 μCi/ml) and infected with* ^{14}*C-labeled wild-type phage or H17 mutant. Lysates were prepared as described in text (Method 3). A 1.5-ml amount of each lysate was layered on top of a neutral sucrose gradient (5–30% [wt/vol]) containing a 2.5-ml CsCl shelf (1.7 g per cm*^{3} *in 40% [wt/vol] sucrose). Centrifugation was at 4 C for 3 h at 25,000 rpm in an SW25 rotor.* ^{14}*C input: am*$^{+}$*, 1,300 counts/min; H17, 900 counts/min.* ^{3}*H input: am*$^{+}$*, 4,600 counts/min; H17, 3,800 counts/min. Recoveries were 86 to 96%. Arrow indicates the position obtained for T4 marker DNA. B, Nonlabeled E. coli B cells were infected with* ^{14}*C-labeled phage as described above. Sedimentation was carried out in alkaline sucrose gradients (5–20% [wt/vol]) at 4 C for 90 min at 32,000 rpm (SW50 rotor). Arrow indicates the position obtained for reference T4* ^{14}*C-DNA released from whole phage and denatured on top of the gradient (15 min in 0.3 N NaOH at 4 C).* ^{14}*C input was 2,700 to 3,050 counts/min. Recoveries greater than 95%.*

though this difference is small, it could be reproduced in many experiments. (In Fig. 3 parental phage DNA cosediments with reference DNA since the lysates were subjected to 0.4% SDS and Pronase digestion [Method 3], whereas in Fig. 1 non-membrane-bound DNA sediments faster since the cells were lysed with 0.1% Sarkosyl and the lysate was not digested with Pronase [Method 1] and probably contained DNA-protein complexes.) Furthermore, it is seen that ^{3}H-labeled *E. coli* DNA has a higher sedimentation coefficient after infection with mutant H17 than with wild-type phage. (The bottom fraction of ^{3}H radioactivity after infection with H17 presumably represents nondegraded or aggregated molecules). The same result was already shown in alkaline gradient analysis (Fig. 2). Comparative analysis of parental DNA at the same time after infection in alkaline sucrose gradients showed (Fig. 3B) cosedimentation of H17 and wild-type DNA with mature phage T4 DNA. Similar results with am^+ phage have been previously described (9, 17). Thus, the difference in sedimentation coefficient between H17 and wild-type DNA observed in neutral sucrose is lost upon denaturation of the DNA.

Properties of T4 DNA synthesized in E. coli B infected with am H17. In order to examine the sedimentation properties of the DNA synthesized in the absence of gene 52 function, *E. coli* B was infected with ^{14}C-labeled T4td8 or H17. Six minutes after infection ^{3}H-thymidine was added, and incubation was continued for 2 or 6 min. Zone sedimentation through alkaline sucrose gradients (Fig. 4) clearly shows that both newly synthesized and parental DNA molecules sediment more slowly at 8 min after H17 infection compared to td8 (considered here as wild type) (Fig. 4A and B). At 8 min after infection, the denatured newly synthesized td8 strands sedimented slowly compared to marker DNA. These results are in accord with previous observations (9). Furthermore, it is apparent that the major portion of parental H17 ^{14}C-DNA has undergone extensive fragmentation from 8 to 12 min after mutant infection (Fig. 4D), while td8 DNA included concatemers (Fig. 4C). In separate experiments, *E. coli* B cells were infected with nonradioactive phage particles, and ^{3}H-thymidine was added from 6 to 12 min after infection with H17 or wild-type phage. The DNA was isolated and sedimented through neutral sucrose gradients (Fig. 5). It can be seen that the bulk of newly synthesized mutant ^{3}H-DNA is highly heterogeneous and sediments at a slower rate than does mature phage T4 DNA (Fig. 5A). In contrast, it is apparent that

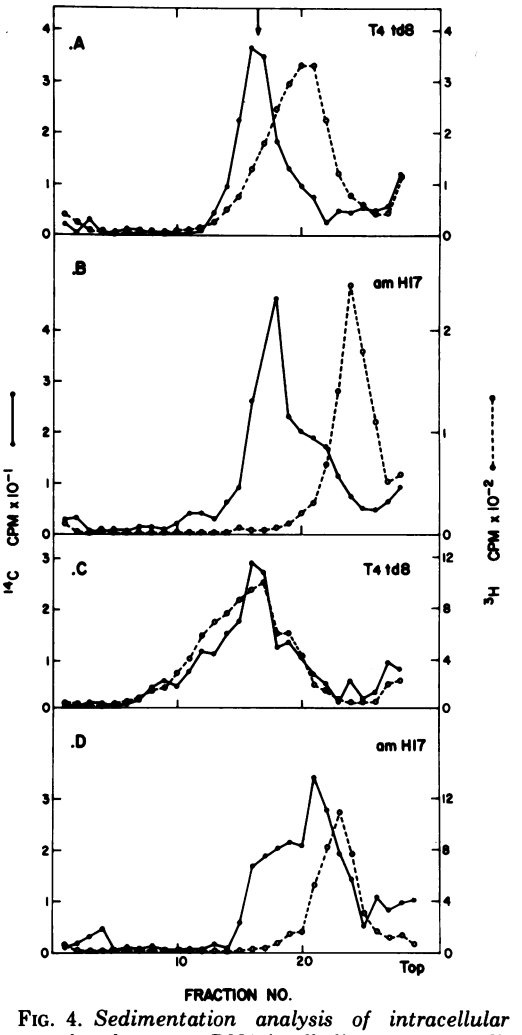

FIG. 4. *Sedimentation analysis of intracellular parental and progeny DNA in alkaline sucrose gradients. E. coli B cells were infected with ^{14}C-labeled T4td8 or mutant H17. Newly synthesized DNA was labeled as described in text (Method 2) by a pulse of ^{3}H-thymidine from 6 to 8 min postinfection (A and B); or from 6 to 12 min postinfection (C and D). Sedimentation was carried out through alkaline sucrose gradients as described in Fig. 3B. Input ^{14}C radioactivity: 246 to 308 counts/min. Input ^{3}H radioactivity: A, 3,600 counts/min; B, 1,800 counts/min; C, 1,200 counts/min; D, 4,300 counts/min. Recoveries were of the order of 80 to 100%.*

wild-type DNA is detected as fast sedimenting replicative form that is heterogeneous in size (Fig. 5B). We conclude that newly synthesized H17 DNA at 12 min postinfection contains double-stranded interruptions (3.2 on the average) and many single-strand breaks which are revealed after denaturation (16.7 on the average).

Pulse-chase experiments. The fate of newly

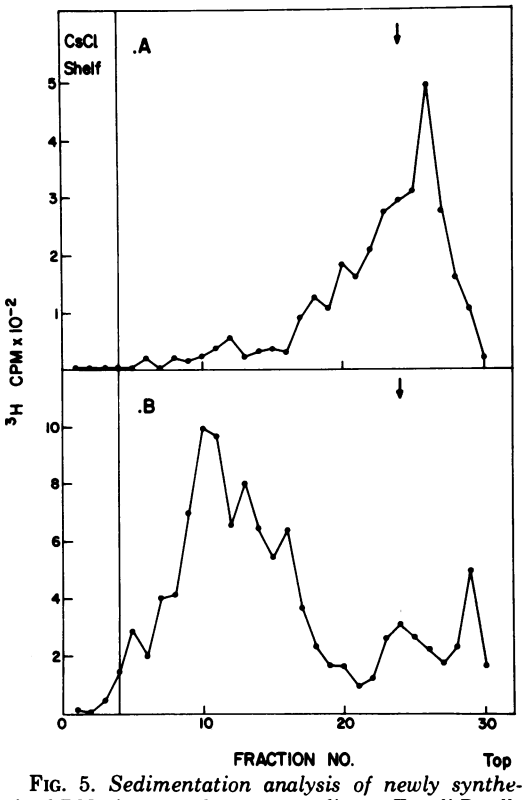

FIG. 5. *Sedimentation analysis of newly synthesized DNA in neutral sucrose gradients. E. coli B cells were infected with nonlabeled mutant H17 (A), or am$^+$ (B). Progeny DNA was pulse-labeled with ^{3}H-thymidine from 6 to 12 min postinfection as in Fig. 4. Sedimentation was carried out through neutral sucrose gradients (5–30% [wt/vol]) containing a 0.8-ml CsCl shelf. Centrifugation was at 4 C for 45 min at 32,000 rpm in an SW39 rotor. The position obtained for T4 DNA released from whole phage is indicated by arrow. Input ^{3}H radioactivity: A, 5,000 counts/min; B, 25,000 counts/min. Recoveries were 80 to 100%.*

synthesized DNA was also observed in pulse-chase experiments, in which ^{3}H-thymidine was added to a culture of T4-infected cells at 6 min postinfection. The culture was allowed to incorporate the labeled precursor for 1.9 min, at which time an excess of unlabeled thymidine was added to the growth medium to prevent further incorporation of ^{3}H. Figure 6 shows the neutral sucrose gradient profile of intracellular T4 DNA-membrane complexes versus chase time. At the start of the chase (8 min), about 66% of the td8 or H17 ^{3}H-DNA was attached to the membrane. At later times (12 min), the proportion of ^{3}H-DNA in this zone remained 66% in case of td8, whereas in H17 most of the ^{3}H-DNA was found on top of the gradient as acid-precipitable, slowly sedimenting material, as could be expected when the attached DNA is detached by endonucleolytic breakage. These findings are in agreement with those shown above (Fig. 4 and 5). However, at a late time after infection (20 min), td8 DNA acquired a sedimentation coefficient close to the mature phage T4 marker (as could be expected when the attached DNA is detached during phage maturation by "headful-cutting" [32]), whereas 54% of ^{3}H-H17 DNA undergoes reattachment to the membrane.

Effect of chloramphenicol on concatemer formation. In view of the extensive fragmentation of parental and progeny mutant DNA, we tried to prevent endonucleolytic breakdown of concatemers by admixture of chloramphenicol (10, 14, 16, 24, 26). It is clearly seen in Fig. 7 that the rate of mutant DNA synthesis as judged by ^{3}H-thymidine incorporation is significantly increased if chloramphenicol is added 10 min after infection (compared to synthesis in its absence), whereas wild-type synthesis is not affected by the addition of the drug at that time. Hence, partial suppression of the "DNA-delay" phenotype is achieved by the addition of the drug 10 min after infection. In an attempt to compare the integrity of ^{14}C-parental and ^{3}H-progeny DNA single strands formed in the presence of chloramphenicol (under conditions of mutant phenotype suppression [Fig. 7]) with single strands formed in its absence, the following experiment was performed. ^{3}H-thymidine was added to a ^{14}C-labeled H17-infected culture at 6 min, followed by chloramphenicol at 10 min, and growth was arrested at 25 min postinfection. The results of alkaline sucrose analysis of ^{14}C-parental and ^{3}H-progeny DNA are shown in Fig. 8. As seen in Fig. 8A, ^{14}C-labeled td8 parental and ^{3}H-labeled progeny DNA have undergone "headful-cutting" (32) by 25 min after infection in the absence of chloramphenicol, whereas in its presence, long single strands are accumulated (Fig. 8C) as a result of inhibition of phage maturation (10). In contrast, mutant parental and progeny DNA molecules are much shorter than genome size (0.26 and 0.11 unit length, respectively, on the average) in the absence of chloramphenicol (Fig. 8B). However, in the presence of the drug (Fig. 8D), concatemeric DNA is formed on mutant infection. We thus conclude that inhibition of endonucleolytic breakage upon addition of chloramphenicol (10) to mutant-infected cells results in increased DNA synthesis on one hand (Fig. 7) and formation of concatemeric DNA on the other.

Nucleolytic activity of H17-infected cell extracts. The extensive fragmentation of parental and newly synthesized DNA molecules in mutant infection described above may result from the presence of a nucleolytic activity.

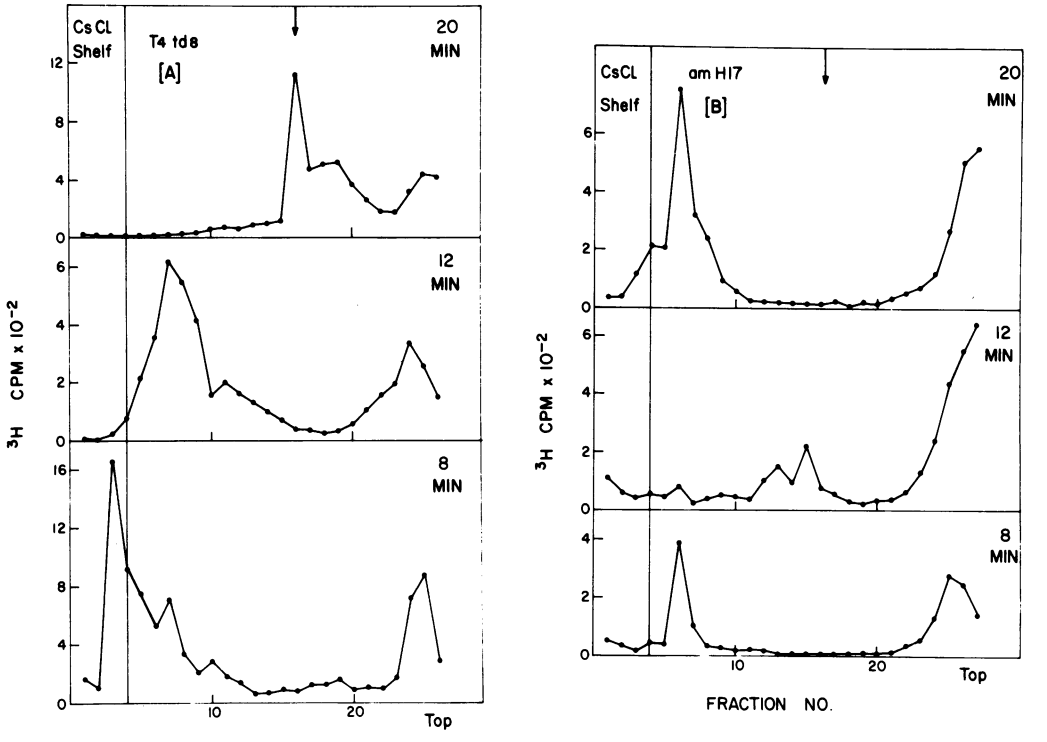

FIG. 6. *Pulse-chase experiment. A culture of E. coli B cells was infected with T4td8 (A) or am H17 (B). At 6 min postinfection, ^{3}H-thymidine (10 μCi/ml) was added to the growth tubes. At 7.9 min an excess of nonlabeled thymidine was added. Samples were withdrawn at 8, 12, and 20 min and lysed as described in text (Method 2). In this experiment the volume of lysate which was applied to the gradient with a wide-tipped pipette varied from sample to sample. Sedimentation was carried out in neutral sucrose gradients (5–30% [wt/vol], containing a 1-ml underlayer of CsCl [1.7 g/cm^3] and 40% [wt/vol] sucrose) for 30 min at 15,000 rpm (SW39 rotor) at 4 C. The position obtained for ^{14}C-labeled whole phage is indicated by the arrow (~1,000S). Total ^{3}H radioactivity was identical in the 8, 12, and 20 min acid-precipitable samples, which showed that the "chase" worked.*

Extracts were therefore examined for the presence of such an activity. (The data presented in earlier sections show a difference between wild type and H17 in nicking to rather large fragments of DNA. Nevertheless, it is possible to assay this activity by acid solubilization of DNA if the H17 nuclease is an endonuclease which introduces single-strand nicks which are then exposed to exonucleolytic digestion.) Extracts were prepared from uninfected cells or from wild-type or H17-infected cells. Table 1 shows the results when T4 sonically disrupted native DNA was treated with these extracts. It is clearly seen that T4 infection of *E. coli* B destroys ATP-dependent deoxyribonuclease activity (33, 39). This activity is also decreased in a gene 52-defective extract; however, under these conditions extensive acid solubilization of T4 DNA occurred independent of ATP. In fact, ATP has an inhibitory effect on this activity. The reason for that is still obscure. These results showed that an activity capable of solubilizing T4 native DNA (ATP-independent) is controlled directly or indirectly by gene 52. This activity is probably not a modified host ATP-dependent deoxyribonuclease because it is found in recombination-deficient bacteria (strain JC4695 RecB$^-$Su$^-$ obtained from A. J. Clark; data not shown). In order to measure this activity in the presence of chloramphenicol, the drug was added to the infected cells 10 min after infection, and the culture was further incubated for 10 min. Addition of chloramphenicol at 10 min caused a 35% decrease in the nucleolytic activity 20 min after mutant infection. The control (no chloramphenicol) at this time (as at 5 min after infection), showed a fivefold increase in mutant extract activity compared to wild type. The inhibitory effect of chloramphenicol on the nucleolytic activity (Table 1) might explain its suppression of the DNA-delay phenotype as shown in Fig. 7, and the integrity of parental and progeny mutant DNA single strands formed in the presence of the drug as

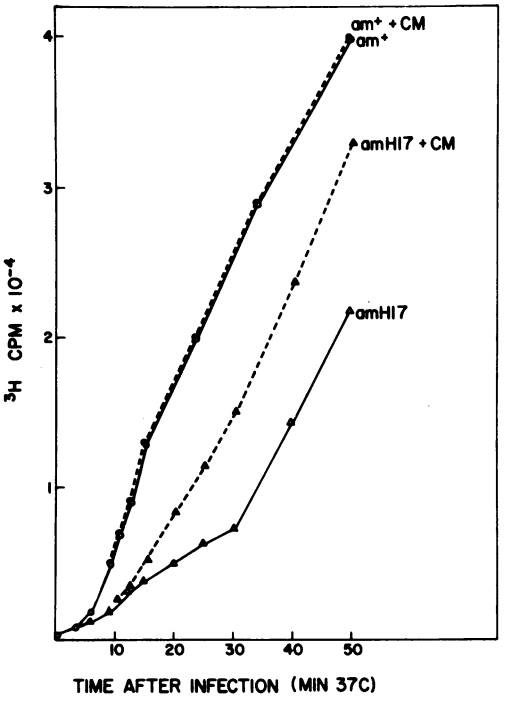

FIG. 7. *Thymidine-methyl-^{3}H incorporation into E. coli B-infected cells. E. coli B cells were grown in Tris-glucose medium to a density of 2 × 10^8 cells per ml. After washing, the cells (2 × 10^8/ml) were infected for 2 min at 26 C with T4am$^+$ (○; ●) or with mutant H17 (△; ▲) at a multiplicity of 10 phage particles per bacterium. At zero time of the experiment, cells were diluted 10-fold into Tris-glucose growth tubes supplemented with thymidine-methyl-^{3}H (1 μCi/ml) and deoxyadenosine (250 μg/ml). At 10 min postinfection, chloramphenicol (30 μg/ml) was added to part of the infected culture (●; ▲). ^{3}H radioactivity was determined by counting 0.5-ml samples after trichloroacetic acid precipitation (29).*

shown in Fig. 8C and D. When the level of activity was measured in extracts of mixedly infected cells (equal input of am^+ and H17 at a MOI = 5), a low nuclease activity typical for wild-type-infected cells was detected, as expected if mutant H17 is recessive to wild type (40). Extract activity could also be measured on sonically disrupted native *E. coli* DNA as substrate. A defect in gene 52 resulted in augmented solubilization (1.7-fold) of this substrate as compared to extract activity of uninfected or wild-type-infected cells. However, at early times after infection in vivo, we showed (Fig. 2) diminished degradation of bacterial DNA after H17 infection. Thus, it seems that, in spite of the presence of the nucleolytic activity in H17-infected cell extracts, the appropriate substrate would not be available in vivo at early times after infection. Hence, we conclude that gene 52 might modify (e.g., by binding to the host membrane) an unrelated T4 or coli function involved in an early state of host DNA degradation.

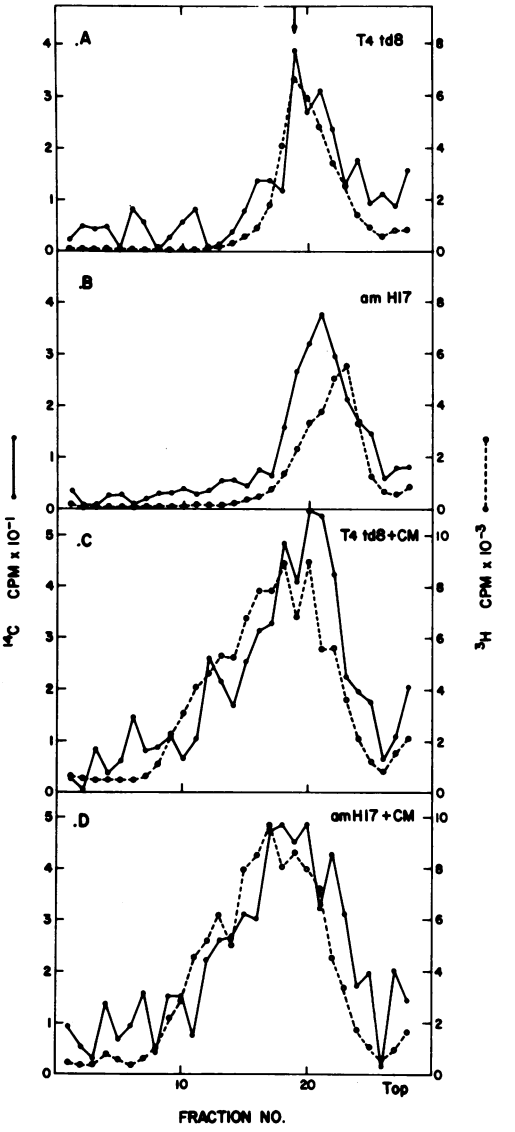

FIG. 8. *Effect of chloramphenicol on concatemer formation. E. coli B cells were infected with ^{14}C-labeled T4td8 (A, C) or mutant H17 (B, D). Newly synthesized DNA was pulse labeled with ^{3}H-thymidine as described in text. At 10 min postinfection, chloramphenicol (30 μg/ml) was added to part of the infected cells (C and D). Growth was arrested 25 min postinfection, and cells were lysed as in Method 3. Analysis through alkaline sucrose gradients was as in Fig. 3B. Input ^{14}C radioactivity: 250 to 350 counts/min. Input ^{3}H radioactivity: A, 56,000 counts/min; B, 13,000 counts/min; C, 70,000 counts/min; D, 35,000 counts/min. Recoveries were 80 to 100%.*

TABLE 1. *T4 DNA breakdown by extracts of T4-infected E. coli B*

Extract	Acid-soluble ^{14}C-DNA (percentage hydrolysis)[a]	
	+ATP	-ATP
Noninfected	22	7
Noninfected + CM[b]	22	5
Wild type	4	4
Wild type + CM[c]	4	3
H17	11	20
H17 + CM[c]	10	13
H17 + wild type[d]	4	4

[a] The value without extract has been subtracted.
[b] Chloramphenicol (CM; 30 μg/ml) was admixed 10 min before sampling.
[c] CM (30 μg/ml) was admixed 10 min after infection. Growth was arrested at 20 min after infection.
[d] Mixed infection, MOI = 5 of each phage strain.

DISCUSSION

Function of gene 52 product. Our results lead to the following conclusions. (i) The attachment of parental T4 DNA to host membrane does not appear to involve gene 52 function, whereas subsequent detachment occurs in its absence. An increase in rate of DNA synthesis later in infection is related to reattachment to the membrane. (ii) With gene 52 product absent, T4 DNA undergoes endonucleolytic breakage. Furthermore, a nucleolytic activity (which does not require ATP) could be detected which is absent in normal infection. In contrast, degradation of bacterial DNA is less extensive following mutant infection. (iii) Formation of concatemers late in infection is conditional on proper functioning of gene 52. In summary, we believe, but do not have sufficient data to establish, that gene 52 product may control the activity of a nuclease in infected cells whose main function may be specific strand nicking in association with DNA replication on one hand and degradation of *E. coli* DNA on the other.

Mechanism of delayed DNA synthesis. After infection with wild-type phage, there is a slow initial rate of DNA synthesis which is gradually increased. However, in DNA-delay mutant-infected cells, the slow rate is not increased until about 30 min postinfection (Fig. 7). Werner (35) suggested that the increase in rate of synthesis is correlated with the increase in the number of growing points. This implies that in mutant infection there is no normal increase in the number of growing points. Furthermore, it has been suggested (4) that multisite initiation occurs at replicative "loops" scattered along parental DNA at an early stage of replication. A slightly higher sedimentation coefficient observed for wild-type DNA 3 min postinfection (Fig. 3A; seen also in Fig. 6C of reference 23), which could not be observed for mutant DNA (Fig. 3A), is compatible with the occurrence of "loops" which are missing in mutant infection (as judged by electron microscopy [19], Y. Naot, D.Sc. thesis, 1972). Since a high multiplicity of infection was used in these experiments, these "loops" might represent initiation sites for DNA replication (4) or intermediates in genetic recombination as suggested for T7 (38).

Since it has been proposed that a nucleolytic activity is involved in initiation of T4 DNA synthesis (11, 12, 17, 35) and since there is some previous evidence for parental strand nicking in association with DNA replication (14, 16, 28), it seems that gene 52 function is essential for proper initiation at early times after infection, whereas at later times it is apparently nonessential. When a second mutation in gene 33 is introduced into a gene 52 mutant (data not shown), no increased replication is observed late in infection. (Similar results were recently described for a gene 39 mutant which was also defective in gene 55 [Fig. 16 in reference 40].) Thus, augmented rate of synthesis in gene 52 mutant-infected cells is probably delayed until specific nicking is performed by later phage functions.

Nonspecific nicking and delayed head formation (40) probably lead to DNA degradation in the absence of gene 52 function. One would expect that DNA-delay mutants in general overproduce "early" nucleases (37) and underproduce the late nucleases (2). However, other DNA-delay mutants tested in gene 39 and 58-61 do not show an overproduction of a nuclease in the assay based on acid-soluble material (Table 1). Thus, these general considerations cannot explain the experimental results. The nucleolytic activity detected in gene 52 mutant infection awaits further characterization.

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