Continued Synthesis of Bacterial DNA After Infection by Bacteriophage T4

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Early in infection by bacteriophage T4, before replication has commenced, one can detect the presence of newly synthesized DNA which cosediments with parental phage DNA on sucrose gradients. As shown earlier (R. E. Murray and C. K. Mathews, 1969), some of this represents covalent attachment of new material to parental phage DNA molecules. However, as shown herein, most of it is bacterial DNA, which is synthesized after infection and presumably degraded to T4 DNA-sized pieces. The small amount of phage-specific DNA synthesis which occurs is apparently a repair process, for its extent is greatly increased if the phage are irradiated with ultraviolet light prior to infection. Analysis by means of pulse labeling with [³H]thymidine and DNA-DNA hybridization shows that host DNA synthesis continues at a significant rate (40 to 80% of the preinfection rate) as late as 10 min after infection at 37 C. Very early in infection this is primarily replicative synthesis, but later a repair process predominates. Presumably this represents attempted repair of damage being inflicted on host DNA by phage-coded nucleases.

As an approach to understanding the initiation of DNA synthesis in cells infected by bacteriophage T4, this laboratory has investigated "prereplicative" events in T4 DNA metabolism—events which occur prior to the onset of phage DNA replication at 5 to 6 min after infection at 37 C. Murray and Mathews (19) showed that, by 4 min after infection, one can detect significant amounts of newly-synthesized DNA, about 0.06 phage-equivalent units per parental phage DNA molecule, which cosediments with T4 DNA on neutral 5 to 20% sucrose gradients. At least some of this "early DNA" represented the addition of nucleotides to parental DNA, as judged by DNA-DNA hybridization and by the fact that early DNA assumed the density of parental DNA. Once formed, early DNA had to maintain its integrity in order for productive infection to occur, because specific inactivation of early DNA led to failure of infected cells to release viable phage (14). Addition of nucleotides to parental T4 DNA in the absence of replication has also been observed by Miller and Kozinski (17).

When early DNA was detected by pulse-labeling with [³H]thymidine followed by sucrose gradient centrifugation, we observed that recoveries of tritium from the gradients were

low, even though we routinely recovered more than 90% of labeled T4 DNA, whether present in radioactive parental phage or in purified material added as a sedimentation marker. This suggested that most of the DNA synthesis early in infection represented continued formation of bacterial DNA, which would sediment to the bottom of the tube under our usual conditions for centrifugation.

Later we observed that the peak of early DNA detected on sucrose gradients did not always coincide with the peak of labeled parental phage DNA, and we wondered whether some of the early DNA represents fragments of bacterial DNA which have been synthesized and partly degraded. (Here and throughout the paper the term "early DNA" refers to any DNA synthesized within the first 5 min of T4 infection and which sediments at approximately the same rate as T4 DNA.) At the same time we were aware that the kinetics of arrest of bacterial DNA synthesis after T4 infection had not been studied in nearly as much detail as the T4-induced shutoff of bacterial RNA and protein synthesis (for reviews see references 6 and 15). The experiments described in this paper were designed to illuminate both of these questions. (Most of this work is taken from a thesis submitted by M. S. Scofield in partial fulfillment of the requirements for a Ph.D. degree in

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MATERIALS AND METHODS

Biological material. *Escherichia coli* strains B, CR63, and B3 have been described in previous publications from this laboratory. *E. coli* 201, like B3, is a low-thymine-requiring derivative of *E. coli* B which lacks thymidylate synthetase. It was kindly provided by G. R. Greenberg and was often used in preference to B3 because of a tendency of the latter strain to clump in minimal media. *E. coli* MX74T2ts27 is a temperature-sensitive mutant which arrests DNA synthesis rapidly upon a shift to 41°C (9). It was kindly provided by A. B. Pardee, *E. coli* 011'(SuI⁺, low thymine-requiring) was supplied by H. Bernstein of the University of Arizona.

T4 phage strains used, including T4D (wild type), T4td8 (thymidylate synthetase-negative), and amN82 (gene 44; DNA-negative) have been maintained in this laboratory for several years. T4nd28 (*denA*; defective in endonuclease II [25]) was kindly provided by H. R. Warner. The double mutant *td8-nd28* was prepared by mixed infection of *E. coli* B with *td8* and *nd28*, and the double mutant *td8-N82* was prepared by mixed infection of *E. coli* B with *td8* and amN82. T4D ghosts were the kind gift of M. Vallée. They were prepared as described elsewhere (see J. B. Cornett, Ph.D. thesis, University of Arizona, Tucson, 1973).

Chemicals. All radioisotopes were purchased from New England Nuclear. Mitomycin C and 5-bromodeoxyuridine (BUdR) were purchased from Sigma Chemical Co. 5-Fluorodeoxyuridine (FUdR) was kindly provided by W. E. Scott and Hoffman-LaRoche. Optical grade CsCl was purchased from Schwarz-Mann Co.

Growth and infection of cells. Bacteria were grown and infected as described in previous papers from this laboratory (12, 13, 16, 19) and are further described in the figure legends. Growth media included M9 (phosphate-buffered glucose minimal medium), TG (Tris-buffered glucose minimal medium), and GCA (glycerol-Casamino Acids medium). Except where otherwise indicated, growth and infection were carried out at 37°C.

Preparation, purification, and assay of phage stocks were as previously described (16, 19), as was the preparation of radioisotopically labeled phage (19). These included ³²P-labeled phage and [¹⁴C]uracil-labeled phage. The latter were prepared by infection in the presence of [2-¹⁴C]uracil, under conditions where over 90% of the pyrimidine bases in phage DNA are derived from exogenous uracil. All ³²P-labeled phages were used within 2 days of preparation, before extensive DNA strand breakage should have occurred.

Pulse labeling of infected cells with [³H]thymidine was performed as described previously (19). In general the isotope was present at 10 μCi/ml of culture and a specific activity of 10 to 20 μCi/mol.

Cell lysates for sedimentation analysis were prepared by the high-temperature Tris-lysozyme-sodium lauryl sulfate method of Frankel (7).

DNA-DNA hybridization. Labeled *E. coli* DNA for hybridization studies was prepared by growth of *E. coli* 201 for one to two generations in GCA medium containing [³H]thymidine at 2 μg/ml and a specific activity of 0.2 μCi/μg. Bacterial DNA, whether from labeled or unlabeled cells, was prepared by phenol extraction (18), as was bacteriophage DNA (23).

Samples of DNA for hybridization were prepared either with phenol or by an unpublished method kindly provided by M. Dinowitz, Department of Microbiology, University of Arizona. Cell lysates prepared by the high-temperature procedure were incubated with 1 mg of Pronase per ml for 3 to 6 h at 37°C. An equal volume of 4% isoamyl alcohol in chloroform was added, and each mixture was rolled at 60 rpm for 30 min at room temperature. The organic phase was removed, and the extraction was repeated. DNA was precipitated from the aqueous phase with 2 volumes of cold ethanol. The precipitate was dissolved in 0.1 × SSC (standard saline citrate), reprecipitated with ethanol, and finally redissolved in 0.1 × SSC. This method gave consistently higher recoveries than methods involving phenol extraction.

DNA prepared as described above was denatured essentially as described by Tomizawa and Ogawa (24). A DNA sample of 0.6 ml was added to an equal volume of 1 N NaOH, and the mixture was heated in a boiling water bath for 7 min. The sample was then neutralized with HCl and adjusted to 3 × SSC and a volume of 3 ml. These preparations then contained about 1 μg each of labeled DNA per ml. This was next annealed to denatured DNA affixed to 0.45-μm membrane filters (Millipore Corp.), by a modification of the method of Denhardt and Burgess (5).

For preparation of immobilized DNA filters, the filters were initially soaked in 6 × SSC and then washed with 10 ml each of 6 × SSC. DNA to be immobilized was diluted such that 20 μg of DNA was present in 3.5 ml of 0.01 × SSC. A series of such samples was heated in a boiling water bath for 10 min, and each sample was adjusted to 6 × SSC by addition of 20 × SSC. Heating was continued for 5 more min, and then each sample was passed through a soaked membrane filter. Retention of DNA on the filter was usually close to 100%, and in those cases where a preliminary experiment showed retention to be incomplete, the initial amount of DNA in each sample was adjusted so that 20 μg of DNA would be immobilized on each filter. The filters were dried in vacuo at 80°C and stored in a desiccator until used.

Prior to measurement of hybridization, each DNA filter was incubated for 6 h at 65°C in 1.5 ml of a mixture of 0.04% each of Ficoll, polyvinylpyrrolidone, and bovine serum albumin in 3 × SSC. During this period the vials containing the filters were shaken at about 50 rpm in a New Brunswick Co. Gyrotory shaker. This procedure reduces DNA binding to blank filters to less than 1% of the input radioactivity.

To each filter, still in the same incubation medium, was added 0.5 ml of denatured labeled DNA sample, each containing about 0.5 μg. Incubation was carried out, with continued agitation, for 12 h at 65°C. The filters were removed, washed on each side with 100 ml of 3 × SSC, dried, and counted in toluene-based

scintillation fluid. Each experiment included control samples of labeled T4 DNA and *E. coli* DNA, and the data in this paper are corrected for the incomplete annealing seen with these samples. In general these control samples of purified T4 DNA hybridized with an efficiency of about 80%, whereas *E. coli* DNA bound with efficiencies close to 20%.

Other methods. Neutral sucrose gradients and CsCl equilibrium gradients were run as previously described (19, 20). UV light-irradiated phage were prepared by illumination, for 30 s, of a sample held 15 cm from a Mineralight lamp. As reported previously (12), this dose corresponds to about 30 lethal hits per phage particle.

RESULTS AND DISCUSSION

Effects of metabolic perturbations on early DNA. As an approach to understanding the molecular nature of T4 early DNA, we explored the effects of various metabolic perturbations upon early DNA synthesis. Figure 1 depicts the results of three such experiments. The experimental design, similar to that described earlier (19), involves labeling of infected cells with [³H]thymidine from 1.5 to 5 min after infection,

followed by gentle lysis and sedimentation through neutral sucrose gradients. As shown in Fig. 1D, E, and F, which represent unperturbed control experiments, most of the ³H label detected in the gradient cosediments with T4 DNA. The amount of this cosedimenting material is greatly decreased if the cells are treated before infection with mitomycin C or if bacterial DNA synthesis is arrested by infection of a *ts* DNA mutant at high temperature (Fig. 1A and B, respectively). The effect of mitomycin is particularly interesting, for pretreatment of cells with this agent does not greatly reduce the total accumulation of phage DNA (Fig. 2). These results both point to an involvement of bacterial enzymes in early DNA synthesis, although they do not distinguish between a bacterial or viral origin for early DNA. Similarly, infection in the presence of chloramphenicol (Fig. 1C) actually increases the amount of labeled DNA which cosediments with T4 DNA, also implicating cellular machinery in the synthesis of early DNA.

Fate of early DNA. As stated earlier, we do

FIG. 1. Early DNA synthesis under different conditions. The results depicted in A and D, B and E, and C and F, respectively, derive from three separate experiments. In each case *E. coli* in M9 medium was infected at a cell density of about 3×10^8 /ml and a multiplicity of infection of 6. [³H]thymidine was added at 1.5 min, and each culture was lysed at 5 min. Samples of 0.2 ml each were centrifuged through 5-ml 5 to 20% neutral sucrose gradients at 40,000 rpm for 60 min (A, B, D, and E) or 90 min (C and F). Sedimentation is from right to left. (A and D) Effect of mitomycin C pretreatment on early DNA synthesis. (A) *E. coli* B3 was treated with mitomycin C (50 μ g/ml) for 10 min, and the drug was removed by centrifugation and washing prior to infection with T4td8. ¹⁴C-labeled phage DNA was added as a sedimentation marker. (D) Identical to A except for the omission of mitomycin C pretreatment. (B and E) Effect of a host cell DNA mutation. *E. coli* MX74T2ts27 was infected with T4td8 at either 41 C (B) or 30 C (E). ¹⁴C-labeled DNA was added as a sedimentation marker. (C and F) Effect of chloramphenicol. *E. coli* B3 was infected with ¹⁴C-labeled T4amN82 in the presence (C) or absence (F) of 100 μ g of chloramphenicol per ml. The left-hand ordinate applies to data in A, B, D, and E, whereas the right-hand ordinate applies to data in C and F.

FIG. 2. Effect of mitomycin C (MC) on DNA synthesis in infected and uninfected cells. *E. coli* B3 was grown in M9 medium to a cell density of about 3×10^8 /ml, and then MC was added to one portion to 50 μ g/ml. Ten minutes later both the treated and untreated samples were centrifuged, washed, resuspended at the same cell density and further subdivided. One pair of cultures was infected with T4td8 at a multiplicity of 6, and the other pair was incubated without infection. In all cases [3 H]thymidine was added at time zero (10 μ g/ml and 10 μ Ci/ml). Samples of 0.1 ml each were removed at the indicated times and applied to filter paper disks which had been presoaked in 5% trichloroacetic acid. The filters were dried, and then washed twice in 5% trichloroacetic acid, twice in acetone, dried, and counted in a Beckman liquid scintillation counter.

not always observe precise cosedimentation of 3 H-labeled early DNA and 14 C- or 32 P-labeled parental or marker T4 DNA. Moreover, the recoveries of 3 H in the gradients are usually low (for example, 17, 22, and 56% in the experiments of Fig. 1D, E, and F, respectively), whereas 70 to 100% of the label in T4 DNA is recovered. We presume that the unrecovered label is in high-molecular-weight bacterial DNA which sediments to the bottom of the centrifuge tube. Partial degradation of this material, a process known to begin within the first few minutes after infection (1, 10, 26), could generate labeled fragments with sedimentation coefficients close to that of T4 DNA.

Experimental support for the above suggestion was obtained in an experiment utilizing 20 to 70% sucrose gradients, which allow recovery of nearly all of the 3 H label. As shown in Fig. 3, continuous labeling was carried out in infection by a gene 44 amber mutant of T4, such that no phage DNA replication could occur. By 4 min nearly all the incorporated label is in rapidly sedimenting material, but by 7 min most of the 3 H cosediments with 32 P-labeled parental phage DNA. However, further degradation of this material occurs, so that by 16 min nearly all of it sediments less rapidly than T4 DNA. About

40% of the acid-insoluble radioactivity present at 4 min has been rendered acid-soluble by 16 min, but virtually all of the acid-insoluble label applied to each gradient was recovered. This experiment also demonstrates formation, and then apparent dissociation, of the "N82 structure," an aggregate of parental T4 *am* N82 DNA molecules which sediments more rapidly than T4 DNA (20). Presence of this material is most apparent at 7 min, although the high viscosity of the 20 to 70% sucrose gradients minimizes the increased sedimentation rate of the parental DNA with respect to mature T4 DNA. However, it is apparent that this structure disaggregates or otherwise changes its conformation, for the parental DNA at 13 and 16 min cosediments with T4 DNA.

Sedimentation of prelabeled bacterial DNA. Direct evidence that bacterial DNA can be degraded to material cosedimenting with T4 DNA was obtained by labeling cells immediately before, rather than immediately after, infection (Fig. 4). Cells were labeled for 2 min with [3 H]thymidine, and then the label was chased by addition of a 200-fold excess of nonradioactive thymidine before infection. As shown in Fig. 4A, a significant amount of this prelabeled bacterial DNA cosediments with T4 DNA when the cells are lysed at 5 min. Phage gene expression is not necessary for this breakdown to occur, since infection with phage ghosts evokes a similar pattern (Fig. 4C). However, active metabolism of the infected cell is apparently required, for infection in the absence of nutrients, or in the cold, greatly decreases the amount of cosedimenting label which is observed (Fig. 4B and D, respectively).

Origin of early DNA. The above data indicate that much of the T4-cosedimenting DNA which we observe at 5 min after infection is actually bacterial DNA, which has been synthesized and partially degraded during the labeling period. This is not expected from our previously published data (19), which suggested that most, if not all, early DNA is viral in origin. This conclusion was based upon (i) the results of DNA-DNA hybridization experiments, (ii) the fact that early DNA assumed the density of parental phage DNA in an experiment using appropriately labeled phage, and (iii) the fact that early DNA contains 5-hydroxymethylcytosine instead of cytosine. The latter point does not constitute strong evidence regarding a viral origin for early DNA. M. J. Hewlett in our laboratory (Ph.D. thesis, University of Arizona, Tucson, 1973) has recently shown that the ability of *E. coli* to recognize and degrade

FIG. 3. Fate of early DNA. *E. coli* B3 in M9 + 2 μg of thymidine per ml was grown to a Klett reading corresponding to about $2.5 \times 10^8/\text{ml}$. The culture was centrifuged and the cells were resuspended in an equal volume of M9 containing no thymidine. A 4-ml sample was infected with ^{32}P -labeled T4amN82 (125 μCi of P per μmol) at a multiplicity of 8. [^3H]thymidine was added immediately to 20 $\mu\text{Ci}/\text{ml}$ of culture. At the indicated times, 0.5-ml samples were withdrawn and lysed. Then 0.2-ml samples of each lysate were layered on 5-ml 20 to 70% neutral sucrose gradients, which were centrifuged for 120 min at 40,000 rpm. The top panel represents the profile obtained when ^{32}P -labeled phage alone were lysed and sedimented, and hence serves as marker for mature T4 DNA. Sedimentation is from right to left. Recoveries of both ^3H and ^{32}P from each gradient were at least 95% of the radioactivity applied. However, the ^3H counts per minute applied decreased from 63,000 in the 4-min gradient to 39,000 in the 16-min gradient, indicating that nearly 40% of the radioactivity incorporated in the first 4 min had been rendered acid soluble by 16 min. Bacterial survival in this experiment was 2%, based on colony-forming ability.

FIG. 4. Coseimentation of prelabeled bacterial DNA with T4 DNA. Each experiment involved growth of *E. coli* 201 in M9 medium plus 2 μ g of thymidine per ml, to a cell density of about 2.5×10^8 /ml. Cells were centrifuged, washed, and resuspended in an equal volume of fresh medium containing no thymidine. The cells were incubated for 10 min, and then [3 H]thymidine was added to 0.4 μ g/ml and 10 μ Ci/ml. After a 2-min labeling period a 200-fold excess of nonradioactive thymidine was added, and 10 min later the cultures were infected as described below. Each infection was terminated at 5 min by lysis of the cells, and lysates were centrifuged in the presence of 14 C-labeled T4 DNA through 5 to 20% neutral sucrose gradients (5-ml gradients; centrifugation for 60 min at 40,000 rpm). (A) Infection with phage at 37 C. (B) Infection with phage in the absence of a carbon source. Immediately before infection the labeled cells were centrifuged, washed, and resuspended in glucose-free medium, and incubated for 10 min further to exhaust nutrient reserves. (C) Infection with phage ghosts at 37 C. (D) Infection with phage at 0 C. Sedimentation is from right to left.

nonglucosylated, hydroxymethylcytosine-containing DNA is abolished after T-even phage infection. Since the dCTP pool begins to be replaced by 5-hydroxymethyl-dCTP quite early in infection (16), one can easily imagine that continued bacterial DNA synthesis after infection would utilize the latter nucleotide, with resultant synthesis of hydroxymethylcytosine-containing bacterial DNA. However, we have reinvestigated the nature of early DNA by the criteria of DNA annealing and density labeling, and the results support the conclusion that a relatively minor fraction of early DNA is phage specific.

Results of a DNA-DNA hybridization experiment are shown in Table 1. Technical improvements in our hybridization procedure have increased its efficiency, and hence its sensitivity, over our previous methods. This experiment involved the simultaneous annealing of 3 H-labeled early DNA and 32 P-labeled parental T4 DNA to DNA (either *E. coli* or T4 DNA)

immobilized on filters. About 35% of the 3 H radioactivity binds to *E. coli* DNA, and 9% binds to T4 DNA. The binding of early DNA to T4 DNA is nearly fourfold higher than the control levels seen when 32 P-labeled T4 DNA is annealed to *E. coli* DNA. When the 3 H counts bound to T4 DNA are corrected for this background of nonspecific binding and also for the incomplete hybridization of 32 P-labeled T4 DNA to immobilized T4 DNA, one can estimate that about 14% of the radioactivity in 3 H-labeled early DNA is in T4-specific sequences.

An unexplained feature of our previously published hybridization data was the failure of early DNA to hybridize with either purified *E. coli* DNA or T4 DNA, although it did hybridize with itself. It also bound to material which we identified as replicating T4 DNA, on the basis of its recovery from fractions near the bottom of a sucrose gradient (7). The present data suggest that binding of early DNA to T4 DNA was simply below the limit of sensitivity of our previous methods. The apparent hybridization to "replicating T4 DNA" can be explained if we assume that the gradient fractions from which this was obtained also contained partially degraded bacterial DNA. We still cannot explain our earlier failure to observe binding of early DNA to *E. coli* DNA, but we have observed such hybridization, with relatively high efficiency, in many experiments other than the one described in Table 1.

Attachment of early DNA to parental DNA. About 1% of 32 P-labeled parental T4 DNA is rendered acid soluble within the first 6 min of infection (20). This is roughly equal to the extent of T4-specific DNA synthesis within this period, if one corrects the amount of early DNA synthesis (0.06 phage-equivalent units per phage genome) for the bacterial contribution. Thus it seems likely that T4 early DNA synthesis represents a repair process, and indeed recent publications from Kozinski's laboratory (3, 11, 22) establish that repair of parental T4 DNA does occur very early in infection. Accordingly, we designed an experiment to (i) confirm our earlier finding that early DNA is attached to parental DNA and (ii) show that the amount of this attached DNA is increased in infection by damaged phage. Results are shown in Fig. 5. Dense, hot phage (labeled with BUdR and 32 P) was used to infect *E. coli*, early DNA was labeled with [3 H]thymidine and then isolated on sucrose gradients, and pycnographic analysis was carried out in a CsCl gradient (Fig. 5B). The same experiment was carried out with phage which were heavily irradiated with UV

TABLE 1. Hybridization of early DNA to T4 and *E. coli* DNA^a

Immobilized DNA	DNA annealed (ml)	³ H early DNA			³² P parental T4 DNA		
		Input counts/min	Bound counts/min	% Bound	Input counts/min	Bound counts/min	% Bound
<i>E. coli</i>	0.3	2,543	904	35.5	1,791	69	3.8
	0.6	5,086	1,779	35.0	3,582	79	2.2
	1.0	8,478	2,836	33.4	5,971	89	1.5
T4	0.3	2,543	222	8.7	1,791	910	50.8
	0.6	5,086	498	9.8	3,582	1607	44.9
	1.0	8,478	716	8.5	5,971	2636	44.1

^a A 3-ml culture of *E. coli* B3 in TGC medium at a cell density of about 3×10^8 /ml was infected on ice with ³²P-labeled T4td8 at a multiplicity of 6. At 5 min the culture was shifted to 37°C, and [³H]thymidine was added to 20 μ Ci/ml. Five minutes later a portion of the culture was plated for determination of bacterial survival (it was 0.5%), and the remainder was lysed at 65°C. Samples of 1 ml each were sedimented through 30-ml 5 to 20% neutral sucrose gradients, and the ³²P-containing fractions were collected by a low-shear method and pooled. This solution, containing ³²P-labeled parental T4 DNA and ³H-labeled early DNA, was denatured as described in Materials and Methods, and samples were annealed separately with filters containing T4 DNA or *E. coli* DNA. In a separate experiment the nonspecific binding of labeled *E. coli* DNA to T4 DNA filters was found to be 2.5% of the input radioactivity.

light immediately before infection (Fig. 5A). In both cases most of the ³H bands at a light density, characteristic of *E. coli* DNA, but a significant amount of ³H bands with the parental ³²P label. The amount of this material is much greater in the experiment with UV-irradiated phage, consistent with the idea that it is the product of repair synthesis. The large amount of light DNA seen in Fig. 5 was not detected in our previously-reported experiment, which was similar in design (19). We cannot explain the discrepancy with our previous results, beyond noting that the type of experiment exemplified by Fig. 5 has been performed several times by each of the present three authors, and we invariably observe large amounts of bacterial DNA synthesis even when host survival is as low as 0.1%.

Rate of host DNA synthesis in T4 infection. The experiments described thus far indicate that considerable host DNA synthesis is still occurring within the first 5 min of infection, but they provide little quantitative information on the total rate of the host DNA synthesis. Published data from several laboratories indicate that host DNA synthesis is shut off soon after infection. In general these conclusions are based upon two types of experiments: (i) measurement of bacterial DNA accumulation in the absence of phage DNA synthesis, either by colorimetric assay or long-term isotopic labeling; and (ii) pulse labeling with DNA precursors, usually with labeled thymidine, often carried out in conjunction with DNA-DNA

hybridization. Measurement of DNA accumulation, of course, can generate negative results if DNA is being degraded concomitantly with its synthesis. Moreover, most of the reported pulse-labeling experiments have used host and phage strains nonauxotrophic for thymidine. As discussed elsewhere (16), the induction of phage thymidylate synthetase and increases in deoxynucleoside triphosphate pools can greatly decrease the rate at which exogenous thymidine is incorporated into DNA. In spite of this, Cascino et al. (4) were able to detect substantial host DNA synthesis in infection by T4 gene 30 mutants (lacking DNA ligase), but they observed considerably less synthesis in cells infected by wild-type phage.

We have investigated host DNA synthesis in T4 infection by pulse labeling with thymidylate synthetase-negative mutants of *E. coli* and T4. This renders the infected cell entirely dependent upon exogenous thymidine for DNA synthesis, and, provided the pulse interval is short with respect to the half-time for DNA breakdown, incorporation data should accurately reflect rates of DNA synthesis. The third pathway for endogenous dTMP generation within a T4-infected cell, namely host DNA breakdown, does not release acid-soluble nucleotides at an appreciable rate until at least 10 min after infection (12). In experiments not shown here, we have found that the half-time for breakdown of host DNA to acid-soluble material is 10 to 15 min in the infected cell. Thus, labeling with 1-min pulses during the first 10 min of infection,

FIG. 5. Association of early DNA with parental phage DNA. Dense, radioactive phage were prepared by infection of *E. coli* 011' in TGC medium containing BUdR at 30 $\mu\text{g}/\text{ml}$ and [^{32}P]orthophosphate at approximately 2×10^{-4} M and a specific activity of 17 $\mu\text{Ci}/\mu\text{mol}$. Because of the relatively low plaque-forming titer of these phages, the titer was estimated from the ultraviolet absorbance of the purified preparation. For the experiment, *E. coli* B3 was grown in GCA medium plus 2 μg of thymine per ml to a Klett reading corresponding to about $3.5 \times 10^8/\text{ml}$, and then centrifuged and resuspended in one-fourth volume of fresh medium. Adsorption of phage, either ultraviolet light-irradiated (A) or unirradiated (B) was carried out in 1-ml cultures chilled in a crushed-ice bath. Approximate multiplicity of infection was 8. After 5 min on ice, 0.25 ml of each culture was added to 0.75 ml of medium containing 30 μCi of [^3H]thymine (about 50 mCi/ μmol), at 37 C. Four minutes later, each culture was lysed, and duplicate 0.3-ml samples of each culture were centrifuged through 5-ml neutral sucrose gradients (90 min at 40,000 rpm). ^{32}P -containing fractions derived from each culture were pooled, dialyzed against SSC, and banded in CsCl gradients. (C) Banding pattern of ^{32}P -labeled DNA from a mixture of light and dense phage. The left-hand peak represents dense DNA, and the right-hand peak represents light DNA. (A and B) The proportion of light ^3H -labeled material (presumably bacterial DNA) to dense material is considerably higher than

followed by hybridization of the extracted DNA samples to T4 or *E. coli* DNA, should yield accurate estimates of the relative total rates of phage and host DNA synthesis. Results of four such experiments are presented in Fig. 6. Figure 6A represents a normal infection of *E. coli* 201 with T4td8, whereas Fig. 6B depicts a synchronized infection, in which phage were admixed with cells at ice temperature and the culture was shifted to 37 C at time zero. Apparently the time needed for temperature equilibration delays metabolic events for a minute or two. Moreover, differences in the experimental protocols have caused variations from one experiment to the next in terms of absolute counts per minute incorporated. However, the following two points are clear, both from the experiments of Fig. 6A and B and from several similar experiments not shown here. (i) There is an early inhibition of host DNA synthesis, followed by an activation nearly to preinfection levels, with the rate at 9 or 10 min after infection still being 40 to 50% of the rate just before infection; and (ii) this late residual host DNA synthesis can be readily detected against a background rate of phage DNA synthesis some 10- to 30-fold higher.

Cascino et al. (4) postulated that continued host DNA synthesis in T4 infection represents an attempt by the infected cell to repair damage to its chromosome which is being inflicted by phage-coded nucleases. Accordingly, one might expect that inhibition of active nuclease synthesis would reduce the amount of postinfection host DNA synthesis. Figure 6C describes an experiment in which *E. coli* 201 was infected with T4td8nd28, a double mutant lacking both thymidylate synthetase and endonuclease II, an enzyme involved in host DNA breakdown (25). However, high levels of residual host synthesis are seen, suggesting that ample sites are still available for the putative repair synthesis. On the other hand, when synthesis of all phage proteins was inhibited by infection in chloramphenicol (Fig. 6D), we note an initial inhibition of host DNA synthesis, with no subsequent increase. As expected, phage DNA synthesis is not activated under these conditions.

Cascino et al. (4) presented evidence implicating DNA polymerase I of the host as a major participant in postinfection host DNA

that seen in other types of experiments. This reflects the inefficient infection of cells by the dense, hot phage (5.1% cell survival in B, 6.4% survival in A). Thus, some of the light DNA represents synthesis in uninfected cells.

FIG. 6. Rate of synthesis of host- and T4-specific DNA in phage-infected cells. Each experiment involved the following general protocol. *E. coli* 201 was grown in GCA medium plus $2 \mu\text{g}$ of thymidine per ml to a cell density of about $3 \times 10^8/\text{ml}$, and then centrifuged and resuspended in an equal volume of the same medium containing thymidine at $0.1 \mu\text{g}/\text{ml}$. Infection with phage, at a multiplicity of about 8 phage particles per cell, was carried out at time zero. At the indicated times (–1 represents 1 min before infection) a 1-ml sample was removed to a tube containing $10 \mu\text{Ci}$ of ^3H thymidine (specific activities ranging from 11 to 18 $\text{mCi}/\mu\text{mol}$ in different experiments). Each pulse labeling was terminated after 1 min, as described in Materials and Methods, and portions of the extracted DNA samples were annealed separately to T4 DNA and *E. coli* DNA. The width of each bar in the figure represents the duration of each labeling period (1 min), and the height represents total radioactivity in each sample hybridizable with either T4 DNA (solid line) or *E. coli* DNA (dashed line). Data are uncorrected for nonspecific binding of T4 DNA to *E. coli* DNA filters, to the extent of about 1% of the input radioactivity. (A) Infection with T4td8. DNA was extracted by the phenol method. Bacterial survival (i.e., relative colony-forming ability at 5 min) was 0.4%. (B) Synchronized infection with T4td8. The culture was infected in a crushed-ice bath at –5 min, and then transferred to a 37°C bath at time zero. DNA was extracted by the phenol method. Bacterial survival was 0.1%. (C) Infection with T4nd28td8. DNA was extracted by the isoamyl alcohol-chloroform method, and the extracts used for hybridization were twofold concentrated with respect

synthesis. However, in one experiment, not shown here, we detected substantial host DNA synthesis in T4td8 infection of a *polA*[–] mutant bacterium. So far we have not investigated the nature of this DNA in detail.

Finally, we should note that the rate of T4-specific DNA synthesis is extremely low within the first 5 min of infection. This agrees with the results of earlier experiments, particularly those of Fig. 5 and Table 1. However, we cannot directly compare the data of Fig. 6 with those of the earlier experiments, because the experiments of Fig. 6 measured total host and phage DNA synthesis, whereas the earlier experiments were concerned only with that DNA which cosediments with T4 DNA.

In comparing our results with those of other laboratories, two points should be kept in mind: (i) as discussed above, our use of thymidylate synthetase-negative strains wherever possible, and (ii) our use of labeled thymidine of very high specific activity. The use of thymineless strains is not essential to detection of host DNA synthesis—for example, the experiment of Fig. 3 was carried out with T4amN82, a *td*⁺ phage—but we believe that this gives better quantitative data. Regarding specific activity, the levels we used are, for example, more than 100-fold higher than those used by Cascino et al. (4), thus facilitating the detection of small amounts of DNA synthesis. Moreover, since Cascino et al. did not report comparable pulse-labeling data for uninfected cells, it is quite possible that the apparently small amount of bacterial DNA synthesis seen in normal infection actually corresponds well with the amounts we detect.

Repair synthesis of host DNA. If host DNA synthesis in T4 infection, particularly that occurring after 5 min, represents repair, then this should be detectable by pycnographic analysis. As shown by Pauling and Hanawalt (21), repair synthesis is nonconservative because the repaired regions are much shorter than the repaired parental and template strands. Accordingly, we grew *E. coli* 201 for two generations in ^{14}C -labeled thymidine and then pulse labeled it with ^3H -BUdR at much higher specific activity. The DNA was then extracted and analyzed on CsCl gradients. Figure 7A shows the results of a control experiment with uninfected cells. Nearly all of the incorporated ^3H bands at a hybrid density position, consistent with the

to the original lysates. Bacterial survival was 0.1%. (D) Infection with *td8* in the presence of chloramphenicol. The drug was added to $100 \mu\text{g}/\text{ml}$ at –5 min. DNA was extracted by the isoamyl alcohol-chloroform technique. Bacterial survival was 0.2%.

Fig. 7. Replicative and repair synthesis of *E. coli* DNA early in T4 infection. *E. coli* 201 was grown for two generations in GCA medium containing [^{14}C]thymidine at 2 $\mu\text{g}/\text{ml}$ and a specific activity of 30 $\mu\text{Ci}/\mu\text{mol}$. The cells were centrifuged, washed, and resuspended at the original density (about $3 \times 10^9/\text{ml}$) in fresh GCA medium. Separate 3-ml samples were labeled with [^3H]BUDr (specific activity 26 $\mu\text{Ci}/\mu\text{mol}$) under the conditions described below. (A) Represents labeling of uninfected cells and depicts control results under conditions where primarily replicative synthesis is occurring. The culture was incubated for 5 min with unlabeled BUDr, to eliminate labeled transition points (8), and then the isotope was added for a subsequent 5-min period. (B) Represents labeling from 1 to 4 min after infection with T4amN82 (multiplicity of infection of 6), whereas (C) depicts an otherwise identical experiment in which the labeling period was 5 to 10 min after infection. In each case labeling was terminated by lysis of the cells, followed by DNA extraction using the chloroform-isoamyl alcohol procedure. Pycnographic analysis was performed in CsCl gradients. A separate analysis of "dense" T4 DNA, fully labeled with BUDr, showed its peak concentration to be in fraction 9 of an otherwise identical gradient.

occurrence of semiconservative replication. Labeling between 1 and 4 min after infection with T4amN82 (a DNA-negative mutant) also gener-

ates a pattern of primarily semiconservative replication, although the peak is skewed toward the light side (Fig. 7B). Much more skewing is seen in results from a 5- to 10-min labeling period (Fig. 7C), in agreement with our hypothesis that much, if not most, of the host DNA synthesis in this period represents repair. It seems likely that much of the ^3H -containing material at the bottom of the gradient, particularly that seen in Fig. 7C, is the RNA-DNA copolymer described by Buckley et al. (2), for this experiment is very similar in design to the experiment of their Fig. 1, which led to a demonstration of the existence of the copolymer.

In summary, early DNA, that which is synthesized in the first 5 min of T4 infection and which cosediments with T4 DNA, is shown to be a mixture of partially degraded host DNA and phage DNA, in the approximate ratio of seven to one. The phage DNA present probably represents repair of parental phage DNA molecules, first because its extent of synthesis equals the amount of material released from parental DNA in the same period, and secondly because its extent is increased severalfold if the phage are irradiated with UV light prior to infection. The synthesis of *E. coli* DNA continues at an appreciable rate for at least 10 min after infection, and much of this synthesis represents attempted repair of virus-inflicted damage. Like preexisting bacterial DNA, synthesized before infection, the newly synthesized material is degraded relatively rapidly, and this may account for the fact that it has not been detected before now.

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