JOURNAL OF VIROLOGY, Aug. 1970, p. 149-155
Vol. 6, No. 2

Printed in U.S.A.

Degradation of *Escherichia coli* B Deoxyribonucleic Acid After Infection with Deoxyribonucleic Acid-Defective Amber Mutants of Bacteriophage T7[1]

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Received for publication 27 April 1970

The degradation of bacterial deoxyribonucleic acid (DNA) was studied after infection of *Escherichia coli* B with DNA-negative amber mutants of bacteriophage T7. Degradation occurred in three stages. (i) Release of the DNA from a rapidly sedimenting cellular structure occurred between 5 and 6 min after infection. (ii) The DNA was cleaved endonucleolytically to fragments having a molecular weight of about 2×10^6 between 6 and 10 min after infection. (iii) These fragments of DNA were reduced to acid-soluble products between 7.5 and 15 min after infection. Stage 1 did not occur in the absence of the gene 1 product (ribonucleic acid polymerase sigma factor), stage 2 did not occur in the absence of the gene 3 product (phage T7-induced endonuclease), and stage 3 did not occur in the absence of the gene 6 product.

It has long been known that *Escherichia coli* deoxyribonucleic acid (DNA) is degraded after infection with T-even (8-10, 12, 14, 15) and T-odd bacteriophages (5, 12, 13, 16, 19). Recently, the breakdown of cellular DNA after infection with bacteriophage T4 has been extensively studied. Mutants defective in genes 46 and 47 fail to degrade host DNA to acid-soluble material (26). Sedimentation studies have suggested that, after T4 infection, *E. coli* DNA first undergoes endonucleolytic breakdown to fragments of DNA with a molecular weight of greater than 10^6 (2, 11, 25). These fragments are then reduced directly to acid-soluble material, a process which requires the action of genes 46 and 47 (11). Two endonucleases which may be involved in the initial stage of degradation of cellular DNA have been purified from T4 phage-infected bacteria (17, 18).

The genetics and physiology of bacteriophage T7 have received considerable attention recently (7, 20, 21). Amber mutants in 19 distinct complementation groups have been isolated and the genes have been ordered on a linear genetic map. The genes are numbered sequentially from left to right, and mutants in genes 1 to 6 are defective in phage DNA synthesis. Gene 1 specifies a T7-induced ribonucleic acid (RNA) polymerase sigma factor which is necessary for subsequent synthesis of most phage proteins (22, 23). Gene 3 is the structural gene for a T7-induced endonuclease involved with breakdown of host-cell DNA (4). It has been suggested that gene 6 codes for an exonuclease which is involved in breakdown of cellular DNA (4). Mutants in gene 1, 3, or 6 do not degrade cellular DNA to acid-soluble material (4, 7).

We have studied the breakdown of *E. coli* B DNA after infection with T7 phages bearing amber mutations in genes 1 to 6. The results reported in this paper show that the breakdown of cellular DNA to acid-soluble material occurs in three discrete stages which require the action of gene 1, gene 3, and gene 6, respectively.

MATERIALS AND METHODS

Bacteria. *E. coli* B, the nonpermissive host for T7 amber mutants, was obtained from J. Wiberg. *E. coli* BBW/1 (7) and *E. coli* 011′ (20), the permissive hosts for T7 amber mutants, were obtained from R. Hausmann and F. W. Studier, respectively.

Bacteriophage. T7 *am*$^+$, T7 *am* H280 (gene 1), and T7 *am* H131 (gene 4) were obtained from R. Hausmann (7), whereas T7 *am* 23 (gene 1), T7 *am* 64 (gene 2), T7 *am* 29 (gene 3), T7 *am* 20 (gene 4), T7 *am* 28 (gene 5), and T7 *am* 147 (gene 6) were the gift of F. W. Studier (20). Double amber mutants were constructed by using the procedure for genetic crosses

[1] Supported by the Medical Research Council of Canada and the S. W. Stedman Foundation.
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described by Studier (20). Wild-type T4D was from the collection of J. Hurwitz.

Chemicals. Unlabeled deoxyadenosine (dA) and deoxythymidine (dT) were purchased from Schwarz BioResearch. Thymine-*2*-^{14}C (59 mCi/mmole) and thymidine-*methyl*-^{3}H (21 Ci/mmole) were obtained from Amersham/Searle. The ^{3}H-thymidine was diluted to a specific activity of 3.0 Ci/mmole with unlabeled thymidine before use. Lysozyme was the product of Worthington Biochemical Corp., and sodium dodecyl sulfate (SDS) was purchased from Sigma Chemical Co.

Growth of bacteriophage. Stocks of T7 amber mutants were grown on the permissive host as described by Hausmann and Gomez (7) or Studier (20). T7^{+} or T4D^{+} DNA was labeled with ^{14}C by growing the phage on *E. coli* BBW/1 whose DNA had been labeled with thymine-*2*-^{14}C. An overnight culture grown in glycerol-Casamino Acids (GCA) medium (6) was diluted 1:50 into GCA medium containing 250 μg of dA per ml and 2 μCi of thymine-*2*-^{14}C per ml. The cells were grown at 37 C to a cell density of about 10^{9}/ml and infected with 0.1 phage per cell. Aeration was continued at 37 C. Cells infected with T7 phage lysed in 40 min, whereas cells infected with T4 phage were lysed with chloroform 4 hr after infection. The phages were purified by differential centrifugation.

Labeling of bacterial DNA. Experiments were done in a reciprocating water bath shaker at 37 C. A 10-ml culture of *E. coli* B was labeled with ^{3}H-dT exactly as described by Kutter and Wiberg (11). Under these conditions, the ^{3}H-dT was incorporated into cold trichloroacetic acid-precipitable material at a linear rate until about 20% of the label had been incorporated. When the cell titer was 3×10^{8} to 5×10^{8}/ml, the cells were washed once in GCA medium and were suspended in 10 ml of GCA medium containing 62.5 μg of dT per ml and 250 μg of dA per ml. After shaking for 2 min at 37 C, the cells were infected with the appropriate T7 phage at a multiplicity of infection of about 10.

Incorpoation of ^{3}H-dT into bacterial DNA and the amount of acid-insoluble radioactivity remaining after infection were followed by precipitating 0.1-ml samples with 5% trichloroacetic acid. Each sample was filtered onto a membrane filter (type HA; Millipore Corp., Bedford, Mass.) or a Whatman 3MM paper disc; the filter was washed with 1% trichloroacetic acid and dried, and the radioactivity was determined in a liquid scintillation counter.

Preparation of cell lysates. The method used was identical to the procedure of Kutter and Wiberg (11), except that 0.05 ml of culture instead of 0.2 ml was used. At a given time after infection, 0.05 ml of infected cells labeled with ^{3}H-dT was withdrawn from the flask and added to a sterile polyallomer centrifuge tube containing an equal volume of a solution of lysozyme (100 μg/ml) in 0.1 M ethylenediaminetetraacetic acid (EDTA), *p*H 8.0. An appropriate amount of ^{14}C-labeled T4 or T7 phage was added to provide a DNA marker of known molecular weight. The mixture was heated to 65 C for 1 min, and SDS was added to a concentration of 0.1%. Incubation at 65 C was continued for 10 min. The lysate was diluted with an equal volume of the phosphate buffer portion of GCA medium containing 0.05 M EDTA before sucrose gradient centrifugation.

Sedimentation analysis. In agreement with Kutter and Wiberg (11), we found that, at early times after infection, the recovery of labeled cellular DNA was poor when the lysate was sedimented in a neutral, 5 to 20% sucrose gradient even if the gradient was formed over a "shelf" of 60% sucrose. This appears to be due to the fact that before infection and early after infection the DNA is bound to a very rapidly sedimenting structure which sediments through the 60% sucrose to the bottom of the tube. When such samples were sedimented through 20 to 60% sucrose gradients at 5 C, about 60% of the radioactivity was recovered in the gradient fractions. An additional 20% could be eluted from the tube with 0.3 N NaOH and appeared to have sedimented to the bottom. Neutral sucrose gradients contained 0.1 M NaCl and 0.05 M EDTA (*p*H 8.5), whereas the alkaline gradients contained 0.7 M NaCl, 0.3 N NaOH, and 10^{-3} M EDTA. The 20 to 60% gradients were 3.2 ml in volume, wheras the 5 to 20% gradients were 3.0 ml in volume and were formed over a 0.2-ml "shelf" of 60% sucrose. For alkaline gradients, 0.1 ml of 0.5 M NaOH was layered on top of the gradient before application of the sample.

The entire lysate (0.2 ml) was applied to the gradient. With 20 to 60% gradients, the lysate was gently poured onto the top of the gradient to minimize shear degradation. The sample was applied to the 5 to 20% gradients with a sawed-off Pasteur pipette. Neutral gradients were formed at 5 C and centrifuged immediately; alkaline gradients were formed at room temperature, and the sample was allowed to stand on top of the gradient for 20 min at room temperature before centrifugation. Centrifugation was done in polyallomer tubes of the IEC SB405 rotor at 35,000 rev/min ($140,000 \times g$) or 54,000 rev/min ($300,000 \times g$) at 5 C. The bottom of the tube was punctured, and the gradient was fractionated into 26 to 29 fractions with a peristaltic pump. To each fraction were added 50 μg of bovine serum albumin and cold 5% trichloroacetic acid. The sample was deposited on a filter disc (Millipore, type HA, or Whatman 3MM paper), which was washed with 1% trichloroacetic acid and dried. Radioactivity was determined with a liquid scintillation spectrometer set for counting ^{3}H in the presence of ^{14}C. The approximate efficiency for counting of the Millipore filters was 35% for tritium and 70% for ^{14}C, whereas for the paper filters the approximate efficiency was 20% for tritium and 40% for ^{14}C. Recoveries of applied radioactivity were greater than 75% for all neutral gradients and better than 90% for alkaline gradients. Approximate molecular weights were determined from the position of the host DNA relative to that of the ^{14}C marker T4 or T7 DNA by using the equation of Burgi and Hershey (3) for neutral sucrose gradients and that of Abelson and Thomas (1) for alkaline sucrose gradients.

RESULTS

Degradation of host-cell DNA to acid-soluble materials. The breakdown of *E. coli* DNA was

studied by measuring the amount of acid-insoluble radioactivity remaining at various times after infection with wild-type T7 and various amber mutants of T7 (Fig. 1). Wild-type T7 caused little loss of acid-insoluble label because the breakdown of host DNA was masked by concomitant synthesis of phage DNA. However, after infection with DNA-negative mutants in gene 4 or gene 5 (7, 20), breakdown of host DNA to acid-soluble material was apparent. This breakdown to acid-soluble material began about 7.5 min after infection and was complete by 15 min after infection. Mutants in gene 1, gene 2, gene 3, and gene 6 caused little solubilization of host DNA. That the apparent limited breakdown caused by the gene 2 mutant is probably due to residual DNA synthesis was shown by the fact that the double mutant (gene 2, 5) showed marked degradation of *E. coli* DNA to acid-soluble material. On the other hand, the double mutants (gene 3, 4 and gene 6, 4) gave identical patterns of breakdown to the single mutants (gene 3 or gene 6, respectively), making it unlikely that the apparent absence of degradation of cellular DNA is due to residual synthesis of T7 DNA. These findings are similar to the results of others (4, 7).

Degradation of E. coli B DNA after infection with DNA-negative mutants (gene 4 or 5). The degradation of bacterial DNA after infection with a gene 4 mutant (*am* H131) was studied by sedimentation analysis in sucrose gradients. The breakdown of cellular DNA has been divided into three stages.

Stage 1. DNA from uninfected bacteria sedimented very rapidly. When the lysate was centrifuged through a 5 to 20% neutral sucrose gradient, the bulk of the labeled DNA sedimented to the bottom of the tube or was trapped in the 60% sucrose "shelf." However, if the lysate was centrifuged through a 20 to 60% neutral sucrose gradient, about half of the DNA was present in a very sharp peak which was about two-thirds of the way down the gradient (Fig. 2a). This position coincided with a distinct white flocculent band which was visible in the gradient. It is possible that this band represents a membranous structure on which SDS has precipitated during the centrifugation at 5 C. The band reached isopycnic equilibrium, since it was in the same position if the gradient was centrifuged at higher speeds than indicated. Five minutes after infection the DNA was still bound to this structure (Fig. 2b). Between 5 and 6 min after infection, the DNA was released from this structure and sedimented at about the same rate as T4 DNA (Fig. 2c).

Stage 2. Endonucleolytic cleavage of the DNA occurred between 7.5 and 10 min after infection (Fig. 2d, e), reducing the DNA from a molecular

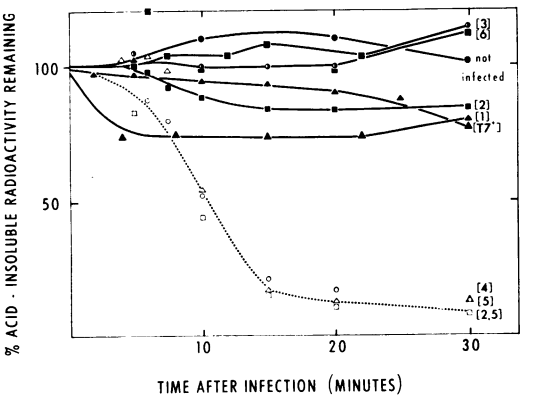

FIG. 1. *Degradation of bacterial DNA to acid-soluble products after infection with various T7 mutants. Cellular DNA was labeled with ^{3}H-dT. The bacteria were infected with the appropriate phage, and the samples removed at given times after infection were precipitated with 5% trichloroacetic acid. Acid-precipitable radioactivity was collected on a filter disc. The numbers in brackets beside each curve refer to the genes(s) bearing the amber mutations.*

weight of about 10^7 to 3×10^7 to about 3×10^6 (Fig. 2d). Between 10 and 15 min after infection, there was not much further breakage of the DNA (Fig. 2f); the remaining acid-insoluble DNA had a molecular weight of about 10^6 to 3×10^6. Comparison of the sedimentation profiles of identical samples from neutral and alkaline sucrose gradients suggested that 7.5 min after infection the fragments of cellular DNA contained some single-strand breaks (Fig. 3a). However, the fragments of DNA isolated 10 and 15 min after infection contained no single strand breaks, since the size of the DNA was comparable in neutral and alkaline sucrose gradients (Fig. 3b, c).

Stage 3. The DNA was converted to acid-soluble fragments (Fig. 1). As shown below, this stage required the action of the gene 6 product.

The same three stages were found to occur in other DNA-negative mutants (gene 5 mutant or gene 2, 5 mutant). This suggests that genes 2, 4, and 5 are not involved in the degradation of cellular DNA after T7 infection.

Effect of a gene 1 mutant on host cell DNA. After infection of *E. coli* B with a T7 phage mutant in gene 1 (T7 *am* H280), there was little change in the banding pattern of labeled cellular DNA when the lysate was spun in 20 to 60% sucrose gradients (Fig. 4a, b). This shows that the release of cellular DNA from a membranous structure (stage 1) is very much delayed in the absence of the gene 1 product. This accounts for the limited breakdown of cellular DNA to acid-

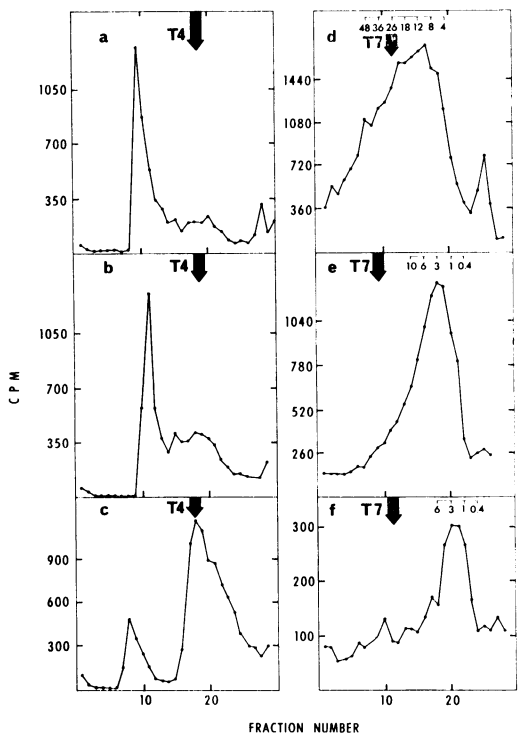

FIG. 2. *Degradation of cellular DNA after infection with gene 4 mutant: neutral sucrose gradients. Cellular DNA was labeled with ^{3}H-dT, and the bacteria were infected with gene 4 mutant phage (am H131). Samples of uninfected cells (Fig. 2a) and of cells 5 and 6 min after infection (Fig. 2b and 2c, respectively) were lysed and layered over 20 to 60% neutral sucrose gradients. The gradients were spun at 140,000 × g for 2.5 hr in the SB405 rotor. Samples taken at 7.5 min (Fig. 2d), 10 min (Fig. 2e), and 15 min (Fig. 2f) after infection were lysed and layered over 5 to 20% neutral sucrose gradients. The gradients were centrifuged at 300,000 × g for 2.5 hr in the SB405 rotor. The position of the ^{14}C-T4 DNA marker is shown by the arrow in Fig. 2a to c. The position of the ^{14}C-T7 DNA marker is shown by the arrow in Fig. 2d to f. The scale at the top of Fig. 2d to f gives the approximate molecular weight in millions as calculated from the equation of Burgi and Hershey (3).*

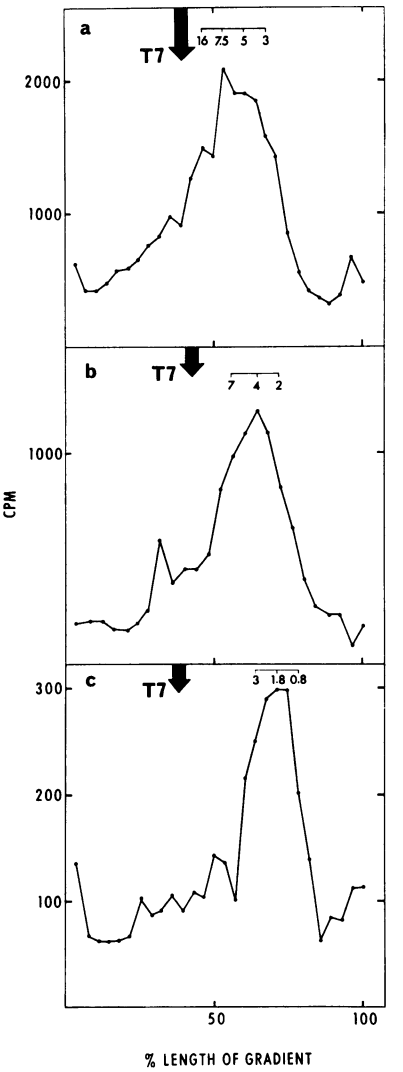

FIG. 3. *Degradation of E. coli DNA after infection with gene 4 mutant: alkaline sucrose gradients. In the same experiment shown in Fig. 2d to f, duplicate samples were taken at 7.5 min (3a), 10 min (3b), and 15 min (3c) after infection, lysed, and layered over 5 to 20% alkaline sucrose gradients. These were centrifuged at 300,000 × g for 2.5 hr. The arrow indicates the position of ^{14}C-T7 marker DNA, and the scale at the top of the diagram shows the approximate single strand molecular weight, in millions as calculated from the equation of Abelson and Thomas (1).*

soluble products after infection with a gene 1 mutant (Fig. 1).

Effect of gene 3 mutant on cellular DNA. Gene 3 is the structural gene for a T7-induced endonuclease which has been implicated in the breakdown of bacterial DNA (4). Indeed, after infection with a gene 3 mutant, the degradation of bacterial DNA was markedly impaired. Although the release of cellular DNA from the rapidly sedimenting structure occurred normally (stage 1), the subsequent endonucleolytic cleavage did not occur (Fig. 5) to any appreciable extent. Even 20 min after infection, the DNA had a molecular weight of about 2×10^{8}. This suggests that the gene 3 endonuclease is responsible for the second stage of breakdown of cellular DNA but is not involved in the initial stage. Furthermore, the DNA cannot be degraded to acid-soluble material without prior endonucleolytic breakdown.

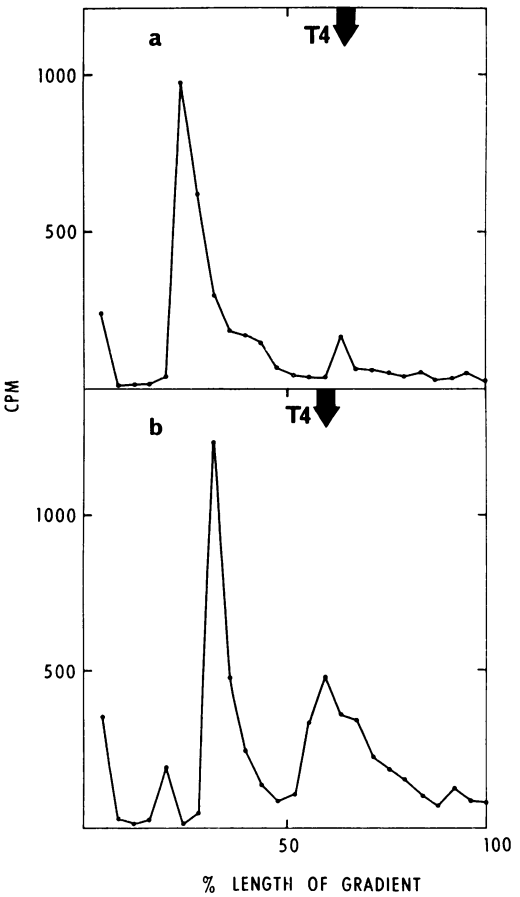

FIG. 4. *Effect of T7 gene 1 mutant on cellular DNA. Labeled E. coli B was infected with T7 am H280 (gene 1). Samples taken immediately before infection (Fig. 4a) and 15 min after infection (Fig. 4b) were lysed and layered over 20 to 60% neutral sucrose gradients. These were spun at 140,000 × g for 2.5 hr in the SB405 rotor. The position of ^{14}C-T4 marker DNA is indicated by the arrow.*

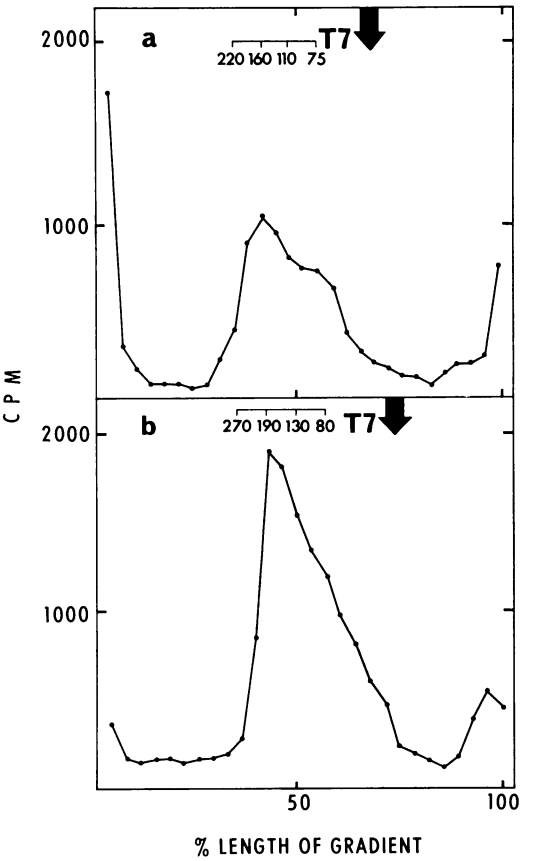

FIG. 5. *Effect of T7 gene 3 mutant on cellular DNA. Labeled E. coli B was infected with T7 am 29 (gene 3). Samples taken 12 (Fig. 5a) and 20 min (Fig. 5b) after infection were lysed and layered over 5 to 20% neutral sucrose gradients. These were centrifuged at 140,000 × g for 2.5 hr. The position of ^{14}C-T7 marker DNA is shown by the arrow. The scale gives the approximate molecular weight in millions.*

Effect of gene 6 mutant on degradation of cellular DNA. After infection with a gene 6 amber mutant (am 147), the first two stages in the degradation of host DNA occurred normally so that by 15 min after infection cellular DNA had been degraded to a molecular weight of about 2×10^6 (Fig. 6). However, as already shown (Fig. 1), this phage caused no appreciable acid-solubilization of host DNA. Hence, we conclude that stage 3, the production of acid-soluble material from cellular DNA, is under the control of the gene 6 product.

DISCUSSION

By studying the sedimentation and banding profiles of cellular DNA after infection with DNA-negative amber mutants of T7, we have divided the process of degradation of host DNA into three stages. The first stage is the release of the DNA from a rapidly sedimenting cellular component as DNA sedimenting at about the same rate as T4 DNA. This stage occurs between 5 and 6 min after infection, and we have been unable to detect the accumulation of significant amounts of DNA sedimenting at a position intermediate between these two forms. We were able to detect this component by centrifuging the lysate in a 20 to 60% sucrose gradient at 5 C. This structure may correspond to the "M band" isolated by Tremblay et al. (24). Their method involved the use of the detergent Sarkosyl and magnesium ions. This resulted in the precipitation of the detergent

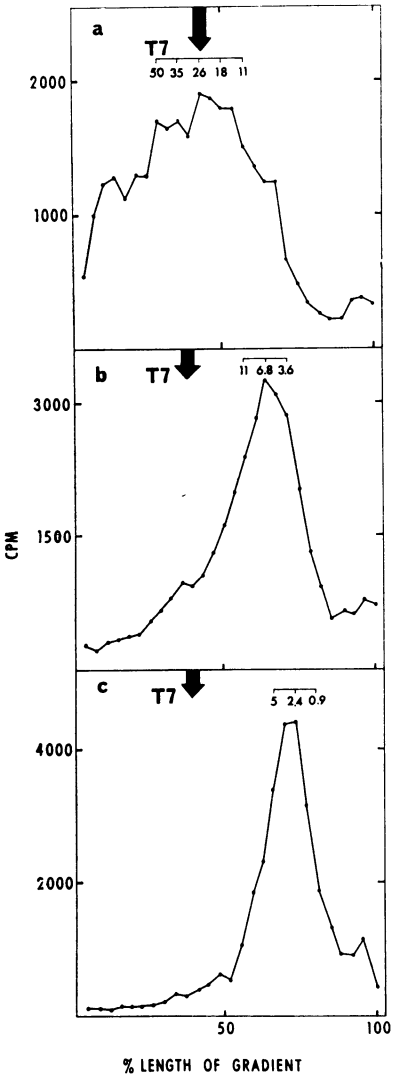

FIG. 6. *Effect of T7 gene 6 mutant on E. coli DNA. Labeled cells were infected with T7 am 147 (gene 6). Samples were taken 7.5 min (Fig. 6a), 10 min (Fig. 6b), and 15 min (Fig. 6c) after infection, lysed, and layered over neutral 5 to 20% sucrose gradients. These were centrifuged at 300,000 × g for 2.5 hr in the SB405 rotor. The position of ^{14}C-T7 marker DNA and an approximate molecular weight scale in millions are shown.*

on the membranous component which could then be banded in a sucrose gradient.

The first stage of degradation requires the function of gene 1. This gene specifies a factor which is needed by the host RNA polymerase to enable it to transcribe the T7 genome (23). Thus, gene 1 mutants make few proteins after infection (22). It is possible that a protein made early after phage infection is necessary to effect the initial stage in degradation of the host genome. However, none of the other mutants examined (genes 2 to 6) seemed to be defective in this stage.

The second stage of breakdown is the endonucleolytic cleavage of cellular DNA to fragments of DNA having a molecular weight as low as 10^6 to 2×10^6. This stage requires the function of gene 3, which is the structural gene for a T7-induced endonuclease (4). The purified enzyme degrades native DNA to a limit product of about 2×10^4 (4), considerably smaller than that observed in the present experiments. In addition, the purified enzyme does not produce significant amounts of acid-soluble material with native DNA. This is consistent with the finding that little acid-soluble material forms after infection with a gene 6 mutant which induces normal levels of the T7 endonuclease.

The third stage of degradation of cellular DNA is the production of acid-soluble material. This stage begins about 7.5 min after infection and proceeds at the same time as the second stage. Ultimately, up to 85% of the cellular DNA is rendered acid-soluble provided that DNA synthesis does not occur. This stage does not occur after infection with a gene 6 mutant, although the second stage (endonucleolytic breakdown) occurs normally. It has been suggested that gene 6 codes for an exonuclease which converts the fragments of DNA produced by the T7 endonuclease to acid-soluble nucleotides (4).

The breakdown of cellular DNA after T7 infection is, in some respects, similar to that found after infection with T4 phage. Both processes involve an endonucleolytic cleavage of the DNA, followed by the conversion of the fragments of DNA to acid-soluble products. The T4-induced endonucleases which have been implicated in the breakdown of cellular DNA after T4 infection do not degrade T4 DNA in vitro, presumably due to the presence of hydroxymethylcytosine instead of cytosine in the DNA (17, 18). In contrast, the T7-induced endonuclease readily degrades purified T7 DNA in vitro (4; P. D. Sadowski, *unpublished data*). Hence, it is unclear why the T7 DNA in the cell is not degraded by the action of the T7 endonuclease.

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