T7 Exonuclease (GENE 6) is Necessary for Molecular Recombination of Bacteriophage T7

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The role of T7-induced exonuclease (gene 6) in molecular recombination was studied by examining the fate of parental DNA during parental-to-progeny recombination. The method used was to compare infections with T7⁺, T7am-6-233 (am gene 6), or T7ts6-136 (ts gene 6) under permissive and nonpermissive conditions. CsCl density gradient analysis of replicative DNA indicated that T7 exonuclease is necessary for recombination to occur, i.e., in the absence of the exonuclease the parental DNA replicated continuously as a hybrid molecule and did not recombine. Further studies under conditions where replicative DNA was denatured and analyzed by CsCl density gradient centrifugation indicated that the exonuclease is also needed for a limited amount of covalent repair of recombinants. A repair function for the T7-induced exonuclease is also suggested by results obtained from alkaline sucrose gradient analysis of replicative DNA. Under conditions nonpermissive for the exonuclease, discontinuities in the DNA accumulated during infection by T7am6-233 or by T7ts6-136.

Bacteriophage T7 participates in molecular recombination to an extent comparable to T4 (20, 26). This makes it an ideal system for studying intermediates and genetic control of recombination.

Molecular recombination can be said to occur in either of two ways: biparental or parental to progeny (13, 15, 20, 28). Parental-to-progeny recombination is examined in a system in which ³²P-labeled parental DNA band in a CsCl gradient at a density heavier than hybrid (13, 16, 17, 20) as a consequence of recombining with 5-bromodeoxyuridine-labeled progeny DNA. There are at least three steps involved in this process: replication of phage DNA, breakage and reassociation of the DNA to form recombinant molecules, and covalent repair of the recombinants. It is well known that phage-coded enzymes are necessary for this phenomenon to occur (2, 16, 17). However, previous to this report, only two genes coding for a characterized product have been correlated with a particular step; T4 DNA polymerase (gene 43) is necessary for covalent repair of T4 recombinant molecules (R. C. Miller, Jr., submitted for publication) and gene 32 product of T4 is necessary for the reassociation stage (14, 29).

Enzymes which might be involved in a diagonal-cut mechanism of recombination (21) could include an endonuclease (nickase), an exonuclease, and an unwinding protein for the breakage and reassociation of the DNA du-

plexes, and a polymerase, exonuclease, and ligase for the repair process. Mutations in genes coding for exonucleases (3, 9, 19, 22) and an unwinding protein (1) are known to affect recombination frequencies or molecular recombination (4, 5, 14, 24, 29) in various systems, but the role of these enzymes is not known.

This report demonstrates that steps similar to those involved in T4 molecular recombination occur in T7 molecular recombination and that the process requires various phage enzymes. Furthermore, it shows specifically that T7 exonuclease (gene 6) is necessary for breakage and reassociation of the DNA and that it also is responsible for a limited amount of the repair of the recombinants.

MATERIALS AND METHODS

Bacteria. All experiments were conducted in *Escherichia coli* B23 (nonpermissive host for amber mutants). O11' is a derivative of *E. coli* B containing an amber suppressor and requiring thymine for growth; it was used for preparation of the amber phage. All bacterial cells were grown for two generations to 3×10^8 cells per ml in H-broth medium prior to resuspension and growth in defined media.

Bacteriophage. Strains of T7 used were a gift from F. W. Studier. The wild-type strain is designated T7⁺. T7am-6-233, an amber in gene 6 which specifies an exonuclease, is designated T7am6. T7ts6-136, a temperature-sensitive mutant of gene 6, is designated T7ts6.

Chemicals and isotopes. L-Chloramphenicol (CM)

and 5-fluorodeoxyuridine were purchased from the Sigma Chemical Company. 5-Bromodeoxyuridine (5-BrdU) was purchased from the Calbiochemical Company. [Methyl-³H]thymidine, 5-[6-³H]bromodeoxyuridine, and ³²P as H₂³²PO₄ were all purchased from New England Nuclear Corporation.

Media. All radioactively labeled phage were prepared, and experiments were performed in TCG media (13; 0.1 M Tris, 0.16 mM Na₂SO₄, 1 mM MgSO₄, 0.05% NaCl, 0.1% glucose, 3 μM FeCl₃, 0.05% vitamin-free Casamino Acids, 0.1 mM CaCl₂, 40 μg of tryptophan per ml, final pH 7.4). TCG was supplemented with phosphate by the addition of 0.1 M KH₂PO₄ as 0.65 ml/100 ml for normal PO₄, TCG, 0.128 ml/100 ml for 1/2 PO₄, TCG, and 6.4 ml/100 for 10 × PO₄, TCG; 10 × PO₄, TCG contains 250 μg of P per ml. 5-BrdU TCG contains 100 μg of 5-BrdU per ml, 5 μg of 5-fluorodeoxyuridine per ml, and 25 μg of uracil per ml.

H-broth contains 5 g of peptone (Difco), 1 g of glucose, 5 g of NaCl, 8 g of nutrient broth (Difco), and 1,000 ml of distilled water, final pH 7.0.

TNE is 0.01 M Tris, 0.015 M EDTA, and 0.15 M NaCl at pH 7.4.

T7 Tris salt is 0.01 M Tris plus 1 M NaCl at pH 7.4.

Preparation of labeled bacteriophage: (i) ³²P. Bacteria were grown for two generations to 3 × 10⁸ cells per ml in [³²P]orthophosphate TCG (at 5 to 10 μCi/μg). Cells were infected with bacteriophage at a multiplicity of infection of 5. Infection by T7⁺ and T7am6 was carried out at 37 C. Infection by T7ts6 was carried out at 30 C. After 90 min, 1 M NaCl was added, the cells were lysed with chloroform, the lysate was treated with DNase, and the phage were purified by differential centrifugation. The phage then were purified further by gel filtration through a column (18 by 0.8 cm) of Bio-Gel A-50M, 100- to 200-mesh (Bio-Rad Laboratories). The column was eluted with T7 Tris salt. Samples (0.5 ml) were collected, and portions were transferred to glass fiber filters, dried, overlaid with toluene-based 1,4-bis-[2]-(5-phenyloxazolyl)benzene and 2,5-diphenyloxazolyl scintillant, and counted for radioactivity in an Isocap 300 scintillation spectrometer.

(ii) ³H. Bacteria were grown in normal phosphate TCG containing thymidine (5 μg/ml), 5-fluorodeoxyuridine (5 μg/ml), and uracil (25 μg/ml). This was supplemented with 1 to 2 μCi of [methyl-³H]thymidine per μg. The bacteriophage were purified as described above.

Heavy-Labeled experimental system. Bacteria were grown in 5-BrdU 10 × PO₄ TCG. Cells were incubated for 2 h in the dark at 30 C and then infected with ³²P-labeled phage at a multiplicity of infection of 7.

Preparation of density-labeled reference T7 DNA. Bacteria were grown in normal PO₄ 5-BrdU TCG for 2 h at 30 C. They were infected at a multiplicity of infection of 5 with T7 phage, and 0.1 μCi of [³H]5-BrdU per μg was added 8 min after infection. CM at 100 μg/ml was added at 15 min after infection to inhibit maturation. Thirty minutes after infection, the reaction was stopped by the addition of

2 volumes of cold TNE. The infective centers were sedimented and resuspended in a small volume of TNE in preparation for the extraction of the DNA. Extracted DNA was dialyzed with a continuous-flow negative-pressure dialysis apparatus against 0.01 M potassium phosphate, pH 7.4, plus 0.001 M EDTA. The density of the progeny DNA was analyzed against ³²P-labeled light T7 DNA by CsCl centrifugation. This DNA was determined to be T7-specific by standard hybridization techniques (10).

Alkaline sucrose gradients. Bacteria infected with ³²P-labeled phage were mixed with ³H-labeled reference T7 phage in TNE; NaOH was added to a final concentration of 0.2 M. This mixture was incubated at 37 C for 20 to 30 min and then layered on a 5-ml 5 to 20% alkaline sucrose gradient. Gradients were centrifuged at 35,000 rpm for 3 h in a Beckman SW50.1 rotor. Between 35 to 40 fractions were collected directly onto glass fiber filters and radioactivity was determined as described above. Silicone-treated polyallomer tubes were used for all sucrose gradients.

DNA extraction and purification. Infective centers in TNE were treated with 0.5% sodium dodecyl sulfate for 5 to 10 min at 37 C and then incubated with predigested Pronase (2 mg/ml) for 5 to 8 h. Samples then were extracted with water-saturated phenol followed by three washes with water-saturated ether. Extraction of DNA from phage was done directly with phenol, followed by washing with ether.

CsCl density analysis. Native ³²P-labeled DNA was analyzed by CsCl density gradient centrifugation: 1.4 ml of sample was mixed with 5 ml of saturated CsCl (in distilled water), or 1 ml of the DNA preparation was mixed with 4 ml of saturated CsCl in silicone-treated polyallomer tubes. The 6.4-ml gradients were overlaid with paraffin oil and centrifuged in an SB283 rotor on a B60 International centrifuge at 5 to 10 C for 48 to 72 h at 30,000 rpm. The 5-ml gradients were centrifuged in an SW50.1 rotor under the same conditions. Preparative CsCl gradients were collected into shell vials in 0.1-ml portions. Aliquots (5 μliters) were transferred to glass fiber filters, and the radioactivity was determined. Replicative bands were isolated from the gradients, dialyzed as described above, and resedimented with both light and heavy ³H-labeled T7 DNA reference in native CsCl gradients. Denatured CsCl gradients were prepared by heat denaturing the DNA from the replicative peaks plus ³H-labeled T7 light reference DNA in the presence of 2% formaldehyde for 5 min at 95 C; the samples then were added to the CsCl in polyallomer tubes treated with denatured DNA. Analytical gradients were collected directly onto glass fiber filters for determining radioactivity.

Determination of label uptake into acid-insoluble material. Samples were pipetted onto 2.5-cm Whatman 3-mm filter disks and washed batchwise in 5% trichloroacetic acid three times for 15 min at 4 C, then twice in anhydrous ethanol. Filters were dried and counted as described above.

Removal of unattached bacteriophage. Bacteria were infected with ³²P-labeled bacteriophage. At 6.5 min after infection, unattached phage were removed

by filtration through a 0.45- μ m millipore filter. Infected cells then were resuspended in an equivalent volume of media which was equilibrated to the experimental temperature.

RESULTS

Recombination with mutants of T7 exonuclease (gene 6). If the T7 exonuclease (gene 6) is required for molecular recombination, then an amber mutant of gene 6 should replicate only to hybrid under nonpermissive conditions. The following experiment was run to test this hypothesis. Wild-type phage (T7⁺) and T7am6 were labeled with ³²P and used to infect separate portions of a nonsuppressing host growing in density-labeling medium (5-BrdU). At a later time after infection, CM was added to inhibit maturation, and the infected cells were incubated to allow substantial replication and recombination. The intracellular DNA was extracted and analyzed by CsCl density gradient sedimentation. Appropriate references were added to determine the position of totally substituted (5-BrdU) and native T7 DNA in the gradients. The pattern of the ³²P label from each gradient is represented in Fig. 1. References from the two gradients overlapped identically as did the conservative DNA (unreplicated parental DNA) from T7⁺ and T7am6. The ³²P label from T7am6 was at a hybrid location, but the T7⁺ replicative DNA was significantly denser than the T7am6, with a large percentage of it heavier than hybrid. Therefore, T7⁺ DNA had recombined, but the T7am6 had not.

Our preliminary conclusion from Fig. 1 is that T7 exonuclease (gene 6) is necessary for recombination, because in its absence the parental DNA replicates only to hybrid. Even though significant DNA synthesis is known to occur with this mutant (25, 26), it is possible that in the absence of the exonuclease not enough replication has taken place to allow recombination, i.e., if the mating pool of intracellular DNA did not include a substantial amount of completely substituted progeny DNA, recombination might not be seen. Therefore, a similar experiment was conducted with a T7 mutant temperature sensitive for the exonuclease (ts6) under conditions which allowed degradation of the host and replication of phage DNA before and after shift to the nonpermissive temperature. *E. coli* were grown in density-labeled medium (5-BrdU), and separate portions were infected with ³²P-labeled T7⁺ or T7ts6. At various times after infection, samples were analyzed in CsCl density gradients. Duplicate samples were incubated further under permis-

FIG. 1. CsCl density sedimentation of ³²P-labeled T7⁺ and ³²P-labeled T7am6 DNA from infected cells. *E. coli* B23 was grown for 2 h at 30 C in the dark in 10 × PO₄ 5-BrdU TCG medium. The culture was split into two parts; one was infected with ³²P-labeled T7 at a multiplicity of infection of 7 and the other was infected at the same multiplicity with ³²P-labeled T7am6. Unattached phage were removed as described. At 16 min after infection, CM (100 μ g/ml) was added and the samples were incubated until 30 min after infection. Samples were added to 2 volumes of cold TNE, sedimented, and resuspended in 1 ml of TNE. The DNA then was extracted and analyzed by CsCl density sedimentation. References were added to determine the position of totally substituted (5-BrdU) and native DNA, and their positions in the gradients are indicated by the arrows. The distributions of ³²P in two gradients are superimposed. The bottom of the gradient is to the left.

sive (30 C) or nonpermissive (43 C) conditions and then were banded in CsCl density gradients. The results of this analysis are represented by Fig. 2A-F. After collection and determination of the radioactivity in the gradients, the replicative DNA was pooled, dialyzed, and reanalyzed in a second set of CsCl density gradients supplemented with completely substituted (5-BrdU) and native reference ³H-labeled T7 DNA (Fig. 2G-L). (Replicative DNA is any DNA displaced from the conservative peak.) Therefore, the experiment compared the extent of replication and recombination of the T7⁺ and the T7ts6 DNA before and after incubation under permissive and nonpermissive conditions. The DNA of both the T7⁺ and T7ts6

FIG. 2. Part I. CsCl density analysis of DNA isolated from cells infected by ^{32}P -labeled T7 $^{+}$ and ^{32}P -labeled T7ts6. *E. coli* B23 was grown for 2 h in the dark at 30 C in $10 \times \text{PO}_4\text{5-BrdUTCG}$. The culture was split into two parts; one was infected with ^{32}P -labeled T7 $^{+}$ at a multiplicity of infection of 7 and the other was infected at the same multiplicity with ^{32}P -labeled T7ts6. Unattached phage were removed as described. At 12 min after infection, three portions of each reaction mixture were taken; one was added directly to 2 volumes of cold TNE, one was added to CM (100 $\mu\text{g}/\text{ml}$) and incubated at 30 C, and one was added to CM and shifted to 43 C. At 30 min after infection, the CM-treated samples were added to 2 volumes of cold TNE. The DNA was extracted and analyzed by preparative CsCl density sedimentation. The distribution of ^{32}P in the gradients was determined. The bottom of the gradient is to the left. (A) T7 $^{+}$ at 12 min; (B) T7 $^{+}$, CM added at 12 min, incubated to 30 min at 30 C; (C) T7 $^{+}$, CM added at 12 min, incubated to 30 min at 43 C; (D) T7ts6 at 12 min; (E) T7ts6, CM added at 12 min, incubated to 30 min at 30 C; (F) T7ts6, CM added at 12 min, incubated to 30 min at 43 C. Part II. CsCl density gradient analysis of replicative DNA. The replicative peaks described in Part I of this figure were isolated and dialyzed against 0.01 M potassium phosphate plus 0.001 M EDTA, pH 7.4, buffer. They were resedimented with totally substituted (^3H)-5-BrdU and light ^3H -labeled T7 DNA references in CsCl density gradients. Gradients were collected directly onto glass fiber filters, and the distribution of both ^{32}P and ^3H in the gradients was determined. The bottom of the gradient is to the left. (G) T7 $^{+}$ at 12 min; (H) T7 $^{+}$, CM added at 12 min, incubated to 30 min at 30 C; (I) T7 $^{+}$, CM added at 12 min, incubated to 30 min at 43 C; (J) T7ts6 at 12 min; (K) T7ts6, CM added at 12 min, incubated to 30 min at 30 C; (L) T7ts6, CM added at 12 min, incubated to 30 min at 43 C.

was replicated to hybrid at 12 min after infection, before shift of temperature (Fig. 2A,D,G,J). Further incubation of the T7 $^{+}$ and T7ts6 at 30 C lead to recombination, i.e., ^{32}P -labeled DNA in all these gradients banded at a

density heavier than hybrid (Fig. 2B,E,H,K). Between 12 and 30 min after infection, both the T7 $^{+}$ and T7ts6 replicated and recombined extensively at the permissive temperature. However, a much different result was obtained

if the infected cells were incubated at the temperature nonpermissive for T7ts6. The wild type recombined as successfully at 43 C as at 30 C (Fig. 2C,I). The DNA was still heavier than hybrid. The T7ts6 DNA did not recombine to a significant extent at the nonpermissive temperature (Fig. 2F,L). By comparing F with C or E or L with K or I, one can see that the DNA in F or L was significantly less dense than their counterparts. The density of T7ts6 DNA had not changed significantly between 12 min (D and J) and after incubation at 43 C (F and L); it remained hybrid. The density of replicated DNA in Fig. 2I (T7⁺ at 43 C) was greater than that shown in Fig. 2H (T7⁺ at 30 C). The replicated DNA from Fig. 2I was also denser than the [³H]5-BrdU reference which was made at 30 C. The DNA polymerase at 43 C may have lost a preference which it had at 30 C for thymidine over 5-BrdU. This result does not affect our conclusions about the necessity of the exonuclease for recombination, because the replicative DNA of the T7ts6 at 43 C did not shift to a recombined density with respect to either heavy reference.

Replication of T7ts6 at 43 C: (i) Uptake of [³H]thymidine. To show that general replication of the T7ts6 DNA was not seriously affected by shift to 43 C, uptake of [³H]thymidine was measured in parallel samples infected with T7⁺ and T7ts6 (Fig. 3). Not only was the uptake of [³H]thymidine to trichloroacetic acid-precipitable material at least as efficient with T7ts6 as T7⁺, but the DNA was phage specific as well. If the exonuclease was inactivated by a temperature shift after T7 DNA replication had been initiated, it did not seem to affect overall DNA synthesis. This supports the conclusion that the exonuclease is directly responsible for recombination and its absence does not stop recombination by elimination of the progeny DNA pool.

(ii) CsCl density gradient analysis. Uptake of [³H]thymidine at a nonpermissive temperature indicated that the exonuclease deficiency did not directly impair the T7 replication apparatus. However, since the pools of available nucleoside triphosphates may be different from wild-type infection after inactivation of the exonuclease, it would be desirable to show directly that the T7 intracellular DNA was replicating at the temperature nonpermissive for the exonuclease. Therefore, an experiment was conducted to show that the intracellular DNA will synthesize a new complement at 43 C. The experiment was done in the following way. *E. coli* were grown in 5-BrdU-labeled medium and infected with ³²P-labeled T7ts6. At 12 min after

FIG. 3. [³H]Thymidine uptake by T7⁺ and T7ts6-infected cultures. *E. coli* B23 was grown to 3×10^8 in $10 \times PO_4$ TCG plus 5-fluorodeoxyuridine (5 μ g/ml) and uracil (25 μ g/ml) at 30 C. The culture was split into two parts; one was infected at a multiplicity of infection of 10 with T7⁺, and the other was infected with T7ts6 at the same multiplicity. At 6 min after infection [³H]thymidine (2 μ Ci/ μ g) was added to each sample, and a portion of each sample was immediately shifted to 43 C. Trichloroacetic acid-insoluble counts were determined for 100- μ l samples at various times after addition of the label. The portions taken at 30 min after infection and incubated at 43 C were extracted, and the purified DNA was hybridized to T7⁺ and *E. coli* B23 DNA. The hybridization data is presented in the inset. I.C., infective center.

infection, the infected bacteria were washed free of 5-BrdU and shifted to 43 C. A sample was lysed, extracted, and analyzed by CsCl density gradient centrifugation to show that the bacteriophage had replicated successfully (Fig. 4A). The rest of the culture was supplemented with thymidine and incubated to 30 min after infection at 43 C. Another sample was lysed, extracted, and analyzed by CsCl density centrifugation (Fig. 4B). Practically all of the label at the hybrid location in Fig. 4A had returned to the light location in Fig. 4B. The ³²P-labeled DNA had exchanged a previously 5-BrdU-

labeled strand for one containing thymidine. The small amount of ^{32}P -label which remained displaced toward the heavy location of the gradient (Fig. 4B) was in excellent agreement with the amount of recombinants found at 12

FIG. 4. Strand exchange at a nonpermissive temperature in cells infected with ^{32}P -labeled T7ts6. *E. coli* B23 was grown for 2 h in $10 \times \text{PO}_4$ 5-BrdUTCG at 30 C. The culture was infected at a multiplicity of infection of 10 with ^{32}P -labeled T7ts6. At 6 min after infection, unattached phage were removed. At 12 min after infection, the infected cells were filtered through a 0.45- μm millipore filter. The filtered cells were resuspended in $10 \times \text{PO}_4$ TCG (containing 5 μg of 5-fluorodeoxyuridine per ml, 25 μg of uracil per ml, and 100 μg of CM per ml) which had been equilibrated to 43 C. Half of the resuspended sample was mixed immediately with 2 volumes of cold TNE; the other half was supplemented with 100 μg of thymidine per ml and incubated at 43 C until 30 min after infection, at which time it also was added to 2 volumes of cold TNE. The DNA was extracted and analyzed by CsCl density sedimentation with admixed light ^3H -labeled T7 DNA. The distribution of ^3H and ^{32}P was determined. The bottom of the gradient is to the left. (A) 12 min after filtration; (B) 12 to 30 min at 43 C.

min after infection (Fig. 2). Since a recombinant has ^{32}P -labeled and 5-BrdU-labeled DNA in the same strand, one would not expect this ^{32}P to return to the light location when thymidine replaces 5-BrdU in the incubation medium. This experiment demonstrates unambiguously that the intracellular T7 DNA was replicating semi-conservatively in the absence of the T7 exonuclease (gene 6), even though it could not recombine.

Repair of recombinants. The following experiment was conducted to see if recombinants formed in the presence of the exonuclease could be repaired in its absence. The procedure was similar to that used in the previous section and described in Fig. 2; the temperature shift was done at a later time, however, to allow recombination before shift to the nonpermissive temperature. Therefore, *E. coli* were grown in a density-labeled medium, and separate portions were infected with ^{32}P -labeled T7 $^+$ or T7ts6. At 14 min, three portions were removed from each culture: one was analyzed by CsCl density gradient sedimentation, the second was incubated further at 30 C, and the third was shifted to and incubated at 43 C. At 30 min after infection, the second and third samples were analyzed by CsCl density gradient analysis also. The gradients were collected and the distribution of radioactivity was determined. Replicative DNA was pooled, dialyzed, and rebanded in CsCl with appropriate references. As expected, all of the DNA was recombined by 14 min and no significant differences were noted in the gradients. However, when the material first was denatured and then rebanded in CsCl, some differences between the T7 $^+$ and the T7ts6 were noted (Fig. 5). Heating the recombinant DNA above the T_m of the DNA disassociated any DNA which was not covalently bonded together. The ^{32}P -labeled DNA of an unrepaired recombinant will return to the light position of the gradient. One can see that at 14 min after infection most of the recombinant DNA was unrepaired (Fig. 5A and D). Further incubation at 30 C lead to extensive repair of the T7 $^+$ and the T7ts6 DNA (Fig. 5B and E). However, incubation of the recombinants at the temperature nonpermissive for the exonuclease (43 C) lead to a noticeable difference between repair of T7 $^+$ DNA and T7ts6 DNA (Fig. 5C and F). If one compares the amount of unrepaired DNA (DNA overlapping with denatured ^3H -labeled T7 DNA), one sees more unrepaired DNA from the T7ts6-infected cells compared to the T7 $^+$ -infected cells. Comparing Fig. 5F with 5C and 5E, one can see less repair with T7ts6 at 43 C

FIG. 5. *CsCl* density gradient analysis of denatured replicative DNA. The same experiment as described in Fig. 2 was repeated using 14-min samples instead of 12-min samples under identical conditions. The samples obtained were extracted, and the DNA was analyzed in preparative *CsCl* density gradients. The distribution of ^{32}P in the gradients was determined. The replicative peaks were isolated, dialyzed, and mixed with light ^3H -labeled T7 DNA, then denatured as described and analyzed by *CsCl* density centrifugation. The distribution of ^3H and ^{32}P in the gradients was determined. The bottom of the gradient is to the left. (A) T7 $^{+}$ at 14 min; (B) T7 $^{+}$, CM added at 14 min, incubated to 30 min at 30 C; (C) T7 $^{+}$, CM added at 14 min, incubated to 30 min at 43 C; (D) T7ts6 at 14 min; (E) T7ts6, CM added at 14 min, incubated to 30 min at 30 C; (F) T7ts6, CM added at 14 min, incubated to 30 min at 43 C.

than the T7 $^{+}$ at 43 C or the T7ts6 at 30 C. In addition, quantitation of the percentage of label found in the covalently repaired recombinant DNA peak indicates that there is an increase in the amount of covalent repair during the T7 $^{+}$ infection at 43 C compared to the same infection incubated at 30 C of about 18%. Comparison of the percentage of covalent repair of the T7ts6 infection incubated at 43 C with incubation at 30 C indicates that there is a 23% decrease in the amount of covalent repair at the higher temperature. This means that in the absence of exonuclease activity, there is a 41% decrease in the amount of covalent repair occurring at 43 C when compared with the wild-type infection under the same conditions. We are forced to conclude that the exonuclease is responsible for a limited amount of repair.

Since the T4 DNA polymerase is responsible for covalent repair of recombinants by filling gaps in the DNA (R. C. Miller, Jr., submitted for publication), one would not expect a great

deal of repair to be mediated necessarily by an exonuclease, but it would be a mistake to conclude that it played no role whatsoever.

Lack of recombination and possibly some covalent repair may be manifested by the following observation: infection of a nonpermissive host leads to an accumulation of discontinuities in the DNA of T7am6. Figure 6 shows that an increasing percentage of the T7am6 DNA is in smaller fragments as infection under nonpermissive conditions continues, i.e., more and more ^{32}P sedimented toward the top of an alkaline sucrose gradient the longer the infected cells were incubated in vivo before analysis. This result can be demonstrated under appropriate conditions with the T7ts6, also. Wild-type phage DNA becomes nicked and then repaired to some degree, but it is not repaired to the same extent as is T4 (16, 21). The accumulation of discontinuities in the T7am6 DNA may reflect the absence of the exonuclease operating on a pre-recombination nick to form a gap. In the absence of recombination, therefore, the nick might not be repaired. This result is consistent with the fact that T4 intracellular DNA develops an extensive amount of very

FIG. 6. Alkaline sucrose gradient sedimentation of intracellular T7am6 DNA. *E. coli* B23 was grown in the dark in $10 \times \text{PO}_4\text{-5-BrdU TCG}$ for 120 min at 30 C, and the culture split into two parts. One part was infected at a multiplicity of infection of 7.0 with ^{32}P -labeled T7 $^{+}$ and one part with ^{32}P -labeled T7am6. At various times after infection CM was added to the samples and the samples were incubated until 30 min after infection. The samples then were analyzed by alkaline sucrose gradient sedimentation. ^3H -labeled T7 phage was added as a reference as described. Sedimentation is from right to left in the figure above. (A) 8-min T7 $^{+}$; (B) 12-min T7 $^{+}$; (C) 16-min T7 $^{+}$; (D) 20-min T7 $^{+}$; (E) 8-min T7am6; (F) 12-min T7am6.

long single strands when recombination is allowed (11, 21), but T7 DNA does not (8, 23).

DISCUSSION

The primary objectives of studies on molecular recombination are to determine the molecular intermediates involved and to be able to attribute the formation of a particular intermediate to a characterized gene product. This report has demonstrated that phage-gene products are essential for molecular recombination of bacteriophage T7. Experiments reported here have shown that the T7 exonuclease (gene 6) is necessary for breakage and reassociation of recombinant DNA.

Genes coding for exonucleases are known to be involved in recombination in several systems (3, 9). Mutations in genes coding for exonucleases frequently decrease recombination frequencies (3, 4, 5, 24). Several published models of recombination invoke the action of exonucleases at one stage or another (7, 9, 27). However, little *in vivo* evidence has been available concerning the particular stage affected by any specific exonuclease.

T7 exonuclease (gene 6) is known to digest double-stranded DNA in a stepwise reaction from a 5' terminus or from an internal single-strand interruption (nick; 12). It is reasonable to assume that, *in vivo*, its activity is to produce a gap which leads to recombination, or that the gap is converted to a sticky end which can anneal with a complementary molecule; alternatively, it may produce sticky ends directly. In either case, it is necessary for the breakage and reassociation stage of recombination.

Results reported here show that the T7 exonuclease (gene 6) is also necessary for a limited amount of covalent repair of recombinants. In view of the known *in vitro* activity of the enzyme (12), it is most reasonable to postulate that it leads to repair through a strand-assimilation mechanism (9). At a recombinant duplex (21), the exonuclease could degrade the complemented strand distal to the whisker, so that the whisker could be assimilated into the duplex and be joined covalently. This does not necessarily imply that the exonuclease must stop exactly at the end of the assimilating strand. DNA polymerase could fill in a gap created by excessive exonucleolytic activity.

Recent studies in our laboratory show that a temperature-sensitive exonuclease (gene 6) causes up to a 10-fold decrease in genetic recombination frequencies at semipermissive conditions of infection (manuscript in preparation).

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