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Purification and Structures of Recombining and Replicating Bacteriophage T7 DNA

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During the infection of *Escherichia coli* by bacteriophage T7, there is a gradual conversion of host DNA to T7 DNA. Recombination and replication occur during this time. We have devised a new way of examining the physical structures of the intermediates of these processes. It is based on the observation that there are no sites in T7 DNA susceptible to cleavage by the restriction endonuclease *Eco*RI. *E. coli* DNA, on the other hand, is susceptible to degradation by *Eco*RI. Thus, phage and host DNA can be separated by sucrose gradient centrifugation after treatment with *Eco*RI. Concatemeric T7 DNA contains a high proportion of branched, gapped, and whiskered structures. These appear to be intermediates of replication and recombination. This approach also monitors the conversion process from host to T7 DNA.

A number of structural features have been related to recombining and replicating bacteriophage DNA. "Eye loops" and forked molecules showing daughter bihelixes of equal length are thought to represent replication intermediates of bacteriophages lambda (6), T4 (3), and T7 (22). Branched DNA, often containing single-chain gaps or "whiskers" (1), has been associated with molecular recombination. The structures predicted by Holliday (5) are also thought to represent recombining molecules and have been observed to arise during colicin E1 recombination (15) and in biparental recombination of T7 phage under conditions in which replication was not permitted (21). The isolation of intermediates during the recombination of T7 is complicated by the presence of host DNA, at least during the early and middle phases of T7 development (20; see below). One way of separating host from T7 sequences is to use a density shift technique with bromodeoxyuridine (8, 17). This approach requires a preparative density gradient and suffers from the disadvantage that some DNA structures may be lost or altered in the CsCl gradient. For example, the large flower-like structures that we recently characterized as intermediates of T7 replication (13) were not observed in the DNA isolated from CsCl gradients after density shift experiments (R. C. Miller and V. Paetkau, unpublished data). In addition, density labeling may impose a physiological perturbation on cells. It is commonly observed that in the presence of bromodeoxyuridine *Escherichia coli* form long, snakelike aggregates.

A novel way of separating T7 sequences from *E. coli* (host) DNA was suggested by the observation that the restriction endonuclease *Eco*RI does not cut T7 DNA (21). Experiments were performed to determine whether host and T7 DNA could be physically separated by sucrose gradient centrifugation after treatment with *Eco*RI. Data will be presented in this paper to show that *Eco*RI can degrade host DNA under conditions in which T7 DNA remains intact. A subsequent centrifugation step separated T7 structures that were of higher molecular weight than unit-sized phage DNA from the degraded residual host DNA. The structures obtained in this way were examined by electron microscopy. Branched and other interesting structures make up a higher fraction of such molecules than was reported recently by another group (17). This is presumably because in the earlier work no separation according to size was carried out.

MATERIALS AND METHODS

Bacteria, phage, and media. *E. coli* B23 were grown in modified M9 medium as described elsewhere (14). All cultures were maintained at 30°C throughout. Cells at a density of between 3×10^8 and 6×10^8/ml were infected with T7 at a multiplicity of 5 to 10. Wild-type T7 and T7 *am*3-29 (endonuclease deficient), designated *am*3, were from F. W. Studier.

Labeling and isolation of intracellular DNA. The method for labeling DNA was described earlier (13). *E. coli* DNA was bulk labeled by adding a total of 1.5 μCi of [^{14}C]thymidine (dThd) per ml (50 mCi/mmol) in several aliquots. The labeling was begun when the cells had reached a density of 2×10^8/ml. The radioactive dThd was added in from 6 to 10 aliquots at 10-min intervals. Under these conditions, radioactive labeling of DNA continues for a few minutes and then stops when dThd is broken down to thymine. Phage DNA was labeled by adding 5 μg of

dThd per ml plus 20 μCi of [^{3}H]dThd per ml (20 Ci/mmol) at 7 min after infection. Under these conditions of excess cold dThd, the uptake of label was linear until about 22 min. This linearity of label incorporation does not, however, reflect the chemical rate of DNA synthesis. To obtain this parameter (see Fig. 1), it is necessary to make chemical measurements and do hybridization tests (see below). Incorporation of labeled dThd was terminated by adding the phenol-ethanol killing solution described earlier (14). Cells were collected by centrifugation and suspended in 100 mM Tris (pH 7.5)–50 mM NaCl–10 mM EDTA in a volume 1/10 that of the original culture. They were incubated for 20 min at 0°C with 0.1 mg of lysozyme per ml and then treated with Sarkosyl, Pronase, and phenol as described earlier (13).

Preparation and use of *Eco*RI restriction endonuclease. This enzyme was prepared essentially by the method of Tanaka and Weisblum (19). The enzyme preparation was at a concentration where 1 μl would cleave 0.52 μg of covalently closed, circular colicin E1 DNA per min at 37°C. A total concentration of 20 mM $MgCl_2$ was added to DNA samples. Then 10 μl of *Eco*RI was added per 0.1 ml of DNA solution. This level was found by titration to be sufficient for the complete degradation of *E. coli* DNA (see Fig. 2). Incubation was at 37°C for 30 min and was quenched by the addition of EDTA to a final total concentration of 30 mM. The sample was then heated to 60°C for 10 min, and 0.2 ml was applied directly to sucrose gradients.

Rate of T7 DNA synthesis. To determine the rate of T7 DNA synthesis, *E. coli* DNA was first bulk labeled with [^{14}C]dThd. The cells were infected with T7 and harvested at various times. The suspended cells were lysed by incubation in 0.3 M NaOH–0.02 M EDTA for 16 h at 37°C. Portions of the infected cultures were also examined directly for acid-insoluble radioactivity at various times. This value declined only slightly during infection, as radioactive *E. coli* DNA was degraded and incorporated into T7 DNA (4, 18). The alkali-treated DNA samples were applied to a 15-m agarose column (0.9 by 30 cm) equilibrated with 0.195 M NaCl–0.01 M potassium phosphate (pH 6.8)–0.2 mM EDTA. DNA emerged at the void volume. It was then heated at dilute concentrations and analyzed by ethidium bromide fluorescence, using denatured *E. coli* DNA as a reference (11). *E. coli* and T7 DNA were found to respond identically in this assay. The specific radioactivity of the DNA and the radioactivity in the infected cells together determine the DNA content per cell as a function of time after infection. The specific activity of intracellular DNA remained the same during the period of T7 DNA synthesis (9 to 17 min). The purified DNA was then analyzed by annealing it to T7 DNA in solution (see below) to determine the fraction of DNA in the sample that was T7. The results of two such experiments are shown in Fig. 1.

Characterization methods. Neutral sucrose gradients were made up and run as described previously (13). All gradients were run in a Beckman SW50.1 rotor at 4°C. The method for carrying out hydroxyapatite analyses for annealed DNA was described previously (12). T7 annealing was driven by having 4 μg of T7 phage DNA per ml present. The results given in this paper were obtained as plateau values between 2 and 3 h of annealing time.

Electron microscopy was carried out as described earlier (13), using the method described by Davis et al. (2).

RESULTS

Rate of conversion of host DNA to T7 DNA. The rate of breakdown of host DNA and the incorporation of the nucleotides into T7 sequences (4, 18) were monitored as described above. Figure 1 shows that there is a rapid phase of T7 DNA synthesis during which time about 60 phage equivalents of DNA are synthesized per cell per min. The bacteria initially contained 280 phage equivalents of DNA. About half of this was converted to T7 DNA by 14 min after infection. Thus, between 12 and 15 min after infection, a significant portion of the intracellular DNA still resides in *E. coli* sequences. As will be shown, this DNA can be of high molecular weight and not directly separable from T7 concatemeric DNA. The uptake of [^{3}H]dThd, which is essentially constant up to about 20 min (17), is not an accurate measure of the absolute rate of DNA synthesis (see below). Under our experimental conditions, lysis occurred at about 30 min after infection.

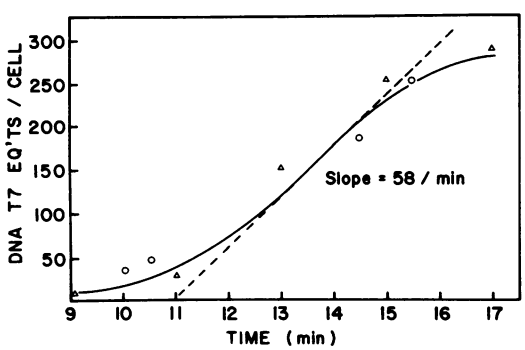

FIG. 1. *Synthesis of T7 DNA in infected E. coli. The DNA of an E. coli culture was uniformly labeled with [^{14}C]dThd, and the cells were suspended and grown for two generations in fresh medium. They were then infected with T7 at a multiplicity of 5. At various times, DNA was purified and tested for T7 sequences by annealing in solution, as described in the text. The specific activity of DNA between 9 and 17 min decreased by 6 to 12%. This does not significantly alter the nature of Fig. 1. The circles and triangles refer to separate experiments. The solid line is a smooth graph of the data. The broken line is an estimate of the maximum slope of this curve and corresponds to the synthesis of about 58 phage equivalents of DNA per cell per min. The total DNA content of the infected cells remained at about 280 phage equivalents of DNA per cell throughout the infection.*

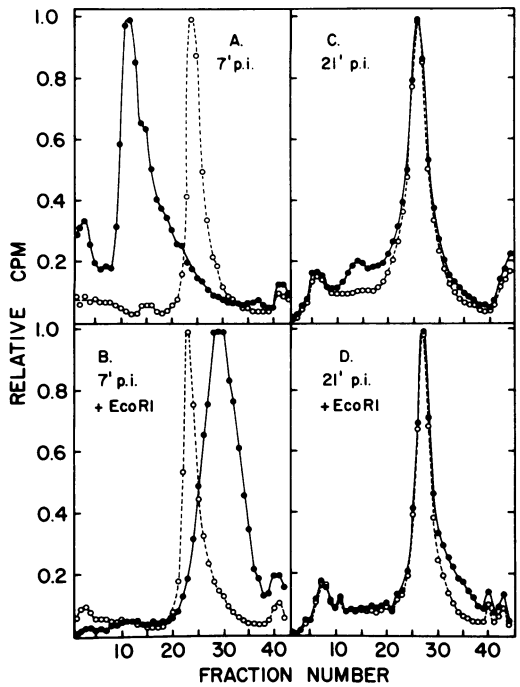

FIG. 2. *Neutral sucrose gradient centrifugation of intracellular DNA. E. coli DNA was uniformly labeled with [^{14}C]dThd before infection. At 7 min after infection, a sample was harvested and the DNA was isolated (A and B). At this time, [^{3}H]dThd was added to the infected culture to label preferentially the bacteriophage DNA being synthesized, and at 21 min after infection a second example of DNA was prepared (C and D). Half of each DNA sample was treated with endonuclease EcoRI, and the remaining half of each sample was incubated in the same salt conditions used for the enzymatic degradation. (B) and (D) represent the EcoRI-treated DNA. A marker of phage DNA (^{3}H labeled) was added to the 7-min sample as a position marker. The 21-min samples ran with the same sedimentation coefficient as marker, unit-sized DNA. Symbols: ●, [^{14}C]DNA; ○, [^{3}H]DNA. Centrifugation was at 45,000 rpm for 2 h at 4°C in an SW50.1 rotor. Sedimentation in all gradients is from right to left. All gradients are expressed relative to the radioactivity in the highest fractions.*

Separation of host and T7 DNA. Experiments were performed to determine whether host and T7 DNA could be physically separated by sucrose gradient centrifugation after treatment with *Eco*RI. Control experiments were first performed to determine under what conditions host DNA would be suitably degraded by this nuclease (Fig. 2). In this experiment, host DNA was labeled before infection with [^{14}C]-dThd. Cells were then infected with phage T7, and at 7 min after infection the DNA was isolated, so that the control would closely mimic the experimental samples. At this time (Fig. 1) there was an insignificant amount of newly synthesized T7 DNA. Figure 2A shows that prelabeled *E. coli* DNA isolated in this way and analyzed in neutral sucrose gradients had a sedimentation value of about 65S. When the same sample of DNA was first treated with *Eco*RI, it was converted to material sedimenting more slowly than unit-sized T7 DNA (Fig. 2B). Late in infection, at 21 min, when essentially all of the host DNA had been converted to T7 sequences, there was little effect of *Eco*RI treatment on the sedimentation profile of either the ^{14}C-labeled bulk DNA or of DNA that had been labeled from 7 min after infection (Fig. 2C and D). Most of the DNA now ran with unit-size T7 DNA. Only a small amount (less than 8% of the total intracellular DNA) behaved as did *E. coli* sequences in its sensitivity to *Eco*RI. Thus, the separation of host DNA from concatemer-sized T7 DNA could be expected by this technique. Since an insignificant amount of the ^{14}C prelabel is lost as acid-soluble material in such an experiment (18), this label serves as a useful indication of the behavior of bulk intracellular DNA. The ^{3}H label added at 7 min after infection is a marker primarily for phage sequences. However, as will be shown, there is a small amount of host DNA synthesized between 7 and 10 min after infection.

The same analysis was performed on samples taken at various intermediate times after infection. Under these experimental conditions (30°C, T7^{+}, M9 medium), the maximal rate of T7 DNA synthesis occurred between 12 and 18 min (Fig. 1). As shown in Fig. 3A and B, only a very small amount of DNA had been converted to T7 by 10 min after infection. The small amount of DNA synthesized at this time apparently included some host sequences (^{3}H label in Fig. 3B). By 15 min after infection, about 50 to 60% of the total DNA (^{14}C label) was in T7 sequences (Fig. 3E and F). The amount of label refractory to *Eco*RI in this experiment agrees approximately with the amount determined by direct hybridization to be in T7 sequences (Fig. 1).

The lack of *Eco*RI-sensitive sites in T7 DNA was confirmed by the data shown in Fig. 3, in which the enzyme had no effect on the fast-sedimenting, flower-like structures of T7 DNA seen during replication (13). Electron microscopic examination showed the fast-sedimenting structures to be unaffected by treatment with *Eco*RI (see also Fig. 5).

To confirm that DNA sedimenting faster than unit-size T7 DNA after *Eco*RI treatment was

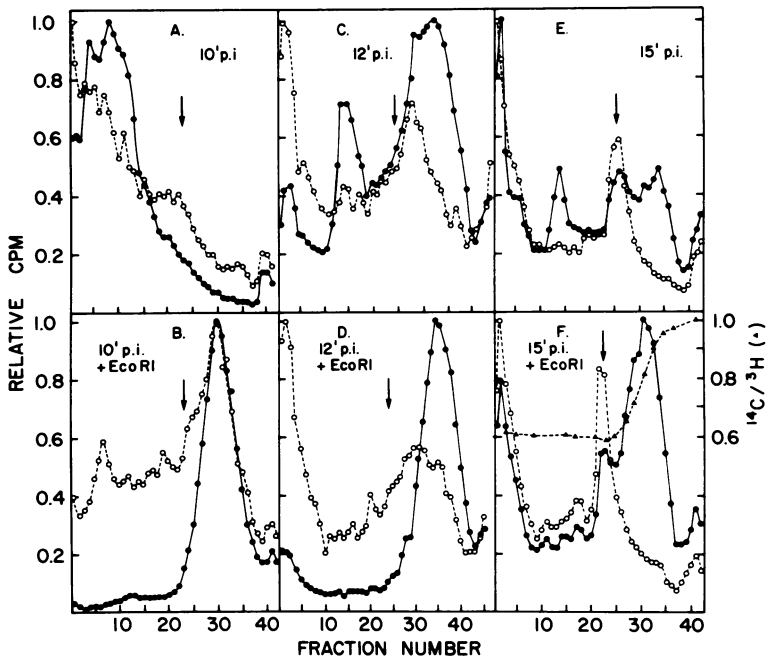

FIG. 3. *Neutral sucrose gradient centrifugation of intracellular DNA at intermediate times. Labeling conditions in this experiment were as described in the legend to Fig. 2. The ^{14}C label (●) was in both E. coli sequences and in newly synthesized T7 DNA. The ^{3}H label (○) added at 7 min was preferentially in newly synthesized phage DNA. Samples were harvested at 10 (A and B), 12 (C and D), and 15 min (E and F) after infection. The arrows indicate the positions of unit-sized phage DNA. (A), (C), and (E) represent DNA untreated with EcoRI; (B), (D), and (F) show the sedimentation profile of DNA treated with EcoRI as described in the legend to Fig. 2 and in the text. In (F), the ratio of accumulated ^{14}C to ^{3}H (▲) is also plotted as a function of position in the gradient beginning at the bottom (left-hand end). This ratio is relatively invariant up to the position of unit-sized DNA and then rises steeply in the area of degraded E. coli DNA sequences. Pooled fractions from the 12-min samples were used in the annealing test for T7 sequences as described in footnote a of Table 1. The fast-sedimenting complex was taken from fractions 1 to 7; the concatemer portion consisted of fractions 9 to 25; and the unit-sized region consisted of fractions 26 to 31. These pools were prepared from both the untreated and the EcoRI-treated samples (C and D, respectively).*

indeed in T7 sequences, we carried out hybridization analyses of various fractions taken from a 12-min sample. Fractions corresponding to the fast-sedimenting complex, to the concatemer region, and to unit-sized T7 DNA were pooled. A quantitative assessment of the ratio of T7 to host sequences is given by hybridization in solution (Table 1). The ^{14}C label reflects the distribution of total DNA in this experiment, whereas the ^{3}H label was incorporated between 7 and 12 min postinfection. There was at this time (12 min) a large content of host (^{14}C) DNA in the complex and concatemer regions (about 48 and 74%, respectively) before *Eco*RI treatment. However, these same regions contained 100 and 85%, respectively, T7 DNA when *Eco*RI digestion preceded sucrose gradient centrifugation. The unit-sized region was still only about 33% T7 DNA even after *Eco*RI treatment.

Analysis of *am*3 mutant DNA. The same analysis was applied to intracellular DNA after infection with T7 bearing an amber mutation in gene 3 (endonuclease), which is used for the degradation of host DNA (18) and conversion of the complex, flower-like T7 DNA structures into concatemers and unit-sized DNA (13) (Fig. 4). It is evident that after infection with *am*3 phage, the process of converting host to phage DNA was much slower than after $T7^{+}$ infection. Indeed, by the time lysis normally occurred under our conditions (30 min), most of the intracellular DNA (^{14}C) was still in host sequences. (Lysis occurred at about 45 to 60 min after *am*3 infection.) Nevertheless, most of the DNA synthesized after about 12 min was in fast-sedimenting T7 structures (^{3}H), as reported earlier (13). This analysis, therefore, also gives a picture of DNA metabolism under various conditions.

Electron microscopy of concatemeric T7 DNA. Perhaps the most useful feature of this

TABLE 1. *Determination of T7 and E. coli sequences in DNA samples at 12 min postinfection*[a]

Pooled sucrose gradient fraction	Annealing of labeled DNA in presence of excess unlabeled T7 DNA (%)		Estimated level of T7 sequences (%)
	^{14}C (preinfection label)	^{3}H (added 7 min postinfection)	
No *Eco*RI treatment			
Fast-sedimenting complex	50	83	52
Concatemers	30	80	26
Unit sized	25	60	19
*Eco*RI-treated before sucrose gradient sedimentation			
Fast-sedimenting complex	87	87	100
Concatemers	75	85	85
Unit sized	35	70	33

[a] Fractions were pooled from sucrose gradients of 12-min samples as indicated in the legends to Fig. 3C and D. The dialyzed and concentrated samples were analyzed for T7 DNA sequences by allowing them to anneal in the presence of T7 DNA, as described in the text. The ^{14}C label was incorporated uniformly into *E. coli* DNA before infection (see Fig. 2 legend and text) and thus represents the total DNA, whereas ^{3}H label represents DNA synthesized between 7 and 12 min postinfection. Thus, to determine the level of T7 sequences in a given sample, the annealing of ^{14}C label was compared with the annealing of authentic *E. coli* and authentic T7 DNA. *E. coli* DNA was 10% double stranded under the conditions used here. A sample of T7 [^{3}H]DNA annealed to the extent of 90% (in a separate tube). This is very close to the observed reannealing of both the ^{3}H and the ^{14}C label of the fast-sedimenting complex after *Eco*RI treatment (87%). Therefore, 87% was taken to represent authentic T7 annealing in this experiment. The fraction of [^{14}C] DNA (bulk) in T7 sequences was then calculated by the formula: fraction in T7 = (^{14}C annealed − 10)/(87 − 10). This number is given in the last column.

approach is to permit the physical isolation of T7 DNA of concatemer or greater size, essentially free of host DNA. Electron microscopic examination of pooled, concatemeric T7^{+} DNA at 12 or 15 min after infection revealed a high proportion of molecules bearing branches, gaps, and whiskers. Typical examples are shown in Fig. 5 to 7. The fraction of concatemeric DNA molecules containing branches, gaps, or whiskers at 12 and 15 min after infection was 30 and 38%, respectively. (The first 100 molecules encountered were examined.) These DNAs were taken from the concatemer regions of sucrose gradients after treatment with *Eco*RI as shown in Fig. 3D and F. Although the hybridization data indicated that up to 15% of this DNA might be in host sequences in the 12-min sample (Table 1), the frequency of branches, gaps, and whiskers was clearly too high to be accounted for by residual host DNA. Of these structures, about half could be rationalized as replication intermediates (Y-shaped molecules or eye loops), but the rest could not. This latter group was similar to T4 recombinant DNA structures (1).

An identically prepared sample from *am*3-infected cells at 15 min after infection yielded only 12% branched, gapped, or whiskered structures. This is consistent with the diminished rate of progeny-related recombination when the gene 3 endonuclease is inactive (7, 9).

The fast-sedimenting, flower-like structures of T7 DNA described earlier (13) normally contain too much DNA to carry out electron microscopic analysis in detail. However, some smaller structures of this type were examined by electron microscopy, and an example is shown in Fig. 5. This complex contains a relatively small amount of DNA, but still resembles the larger, flower-like structures by having a central core. A number of branch points can be seen in this structure, which was isolated from sucrose gradients after

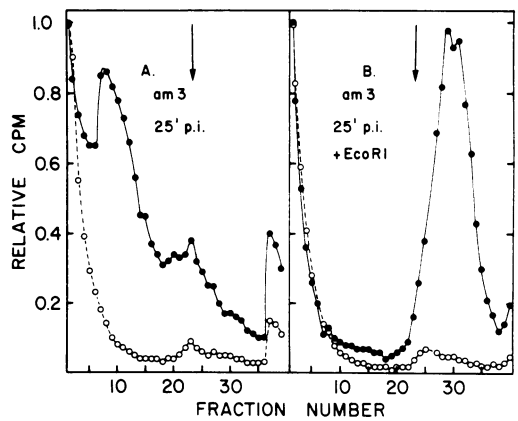

FIG. 4. *Neutral sucrose gradient analysis of intracellular DNA after infection with T7 am3. A culture of E. coli cells was prelabeled with [^{14}C]dThd and infected with T7 am3. At 8 min after infection, [^{3}H]dThd was added. Samples were harvested at 25 min after infection, worked up, and analyzed by sucrose gradient centrifugation as described in the legends to Fig. 2 and 3. The arrows indicate the position of unit-sized T7 DNA in these gradients. Other conditions were as described in the legend to Fig. 2. The sample analyzed in (B) had been treated with EcoRI. Symbols: ●, ^{14}C label; ○, ^{3}H label. The large peak of [^{14}C]DNA running more slowly than the unit-sized marker in (B) represents E. coli DNA that has not been converted to T7 sequences.*

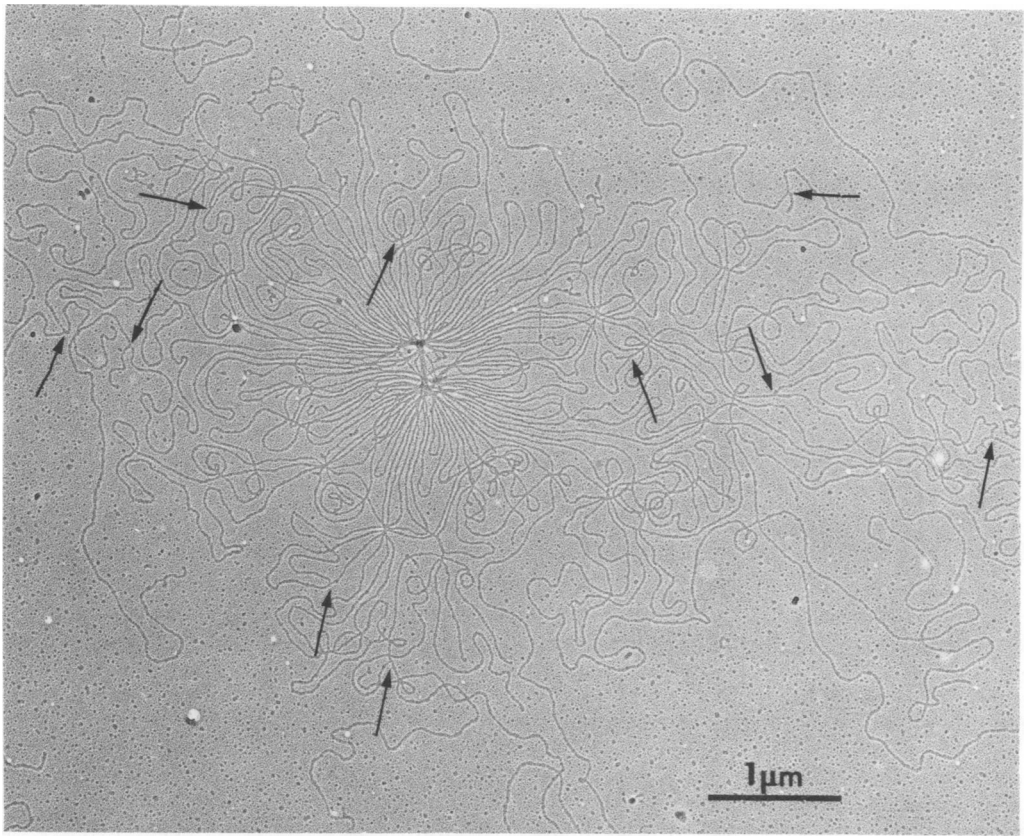

FIG. 5. *Electron microscopy of the fast-sedimenting (flower) form of T7 DNA isolated after treatment with EcoRI. A sample of T7⁺ DNA was isolated at 15 min postinfection, treated with EcoRI endonuclease, and run through a neutral sucrose gradient. The concatemer region, extending to the edge of the fast-sedimenting complex zone, was examined by electron microscopy. The flower-like structure is similar to, although smaller than, the complexes described earlier (13). It was presumably present in this sample because of its relatively lower DNA content. The arrows indicate branches seen in this structure and assumed to arise from replication or recombination (see text).*

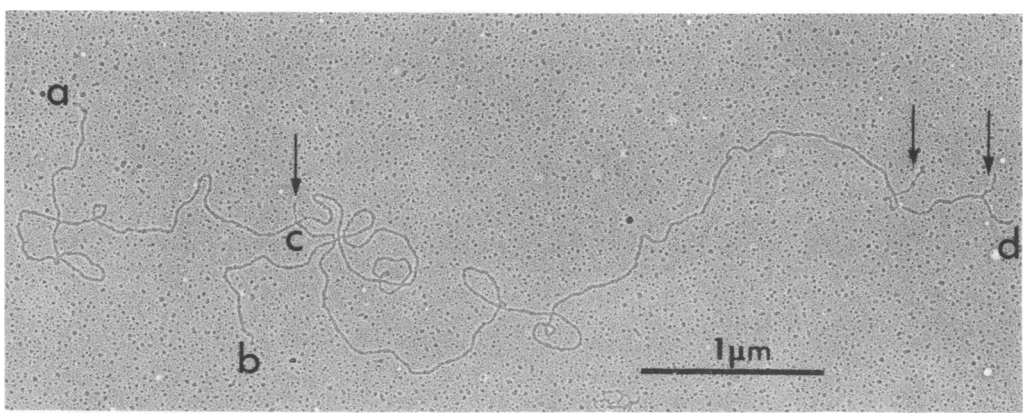

FIG. 6. *Example of branches and a whisker in a replicating T7⁺ DNA molecule. This structure was seen in a sample of EcoRI-treated intracellular DNA taken at 15 min after infection and sedimenting faster than unit-sized phage DNA. The distances a-c and b-c are 4.62 and 4.64 μm, respectively, and the distance c-d is 7.72 μm. Thus, both a-d and b-d are 12.3 μm, or one phage equivalent. The region at c is therefore undoubtedly a replication fork, and the whisker at c is a single-stranded, newly synthesized chain, presumably the 3′ end, as found in replicating T4 DNA by Delius et al. (3). The other arrows near d may show single-stranded recombination branches.*

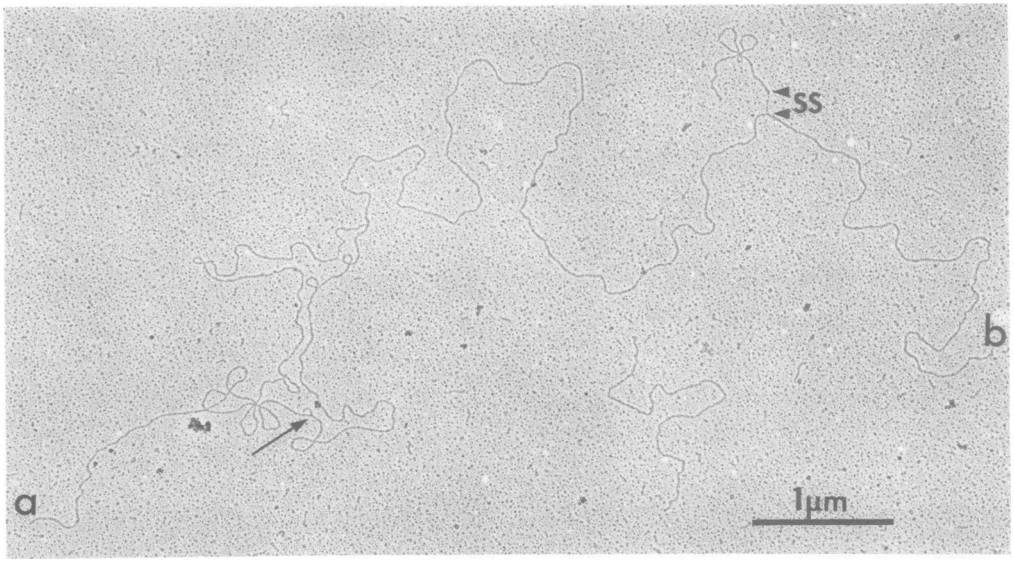

FIG. 7. *Branches in replicating T7⁺ DNA. This sample was prepared in the same way as the molecule shown in Fig. 6. At the position marked ss, a single-stranded gap connects double-stranded branches. The eye loop (22) has branches of about 5.6 μm each (44% of a phage equivalent). The distance a-b is 1.6 phage equivalents. The arrow points out what appears to be a very short branch within the eye loop.*

treatment with *Eco*RI to remove *E. coli* DNA.

Figures 6 and 7 show examples of the replicating eye-loop structures (22). In Fig. 6, the replicating molecule shown is one phage equivalent long, and in Fig. 7 the total length shown is 1.4 or 1.6 phage equivalents, depending on the branch taken. Figure 6 shows a short, single-stranded whisker (3) at one replication fork, as well as two short, single-stranded branches.

DISCUSSION

The primary objective of this work was to examine the structure of intracellular T7 DNA of greater than unit length, using a technique that would not perturb the existing structures. Previous work from this laboratory (13, 14) demonstrated that intracellular T7 DNA could be quantitatively recovered after the exhaustive deproteinization procedure used in all of this work (lipid would probably also be removed). The evidence presented here indicates that branched, gapped, and whiskered structures are associated with normal T7 DNA in progeny-related replication and recombination. The isolation of such structures is possible by the combination of treatment with *Eco*RI nuclease and isolation from preparative sucrose gradients. The relatively high frequency of such structures seen here (30 to 38% of concatemeric molecules) is a result of enrichment by these techniques. These figures are higher than ones reported earlier for such DNA isolated by density shift techniques (17). In the earlier report a yield of only about 12% branched molecules was observed at 15 min after infection. Some of the structures seen in this work (Fig. 6 and 7) are apparently replication intermediates. The evidence for this is that they have equal-length arms in an eye-loop structure. One example shown in Fig. 6 also has a short, single-stranded whisker protruding at the replication fork. This is similar to the structures seen by Delius et al. (3) and probably arises by branch migration during the isolation of the structures. This type of branch migration apparently also drives out short, metastable fragments from the T7 replication fork (14). Other structures cannot be rationalized by simple replication mechanisms and presumably arise from recombination. The occurrence of double-stranded branches in the fast-sedimenting, flower-like structure shown in Fig. 5 is consistent with the role of this type of structure in replication (13).

The DNA structures shown in Fig. 5 to 7 contain unit-sized, larger-than-unit-sized, and highly concatemeric DNAs. In each of these examples, there exist short branches, which are probably a reflection of molecular recombination. The existence of short branches in the flower-like structure suggests that molecular recombination occurs within this structure as well as in concatemeric or unit-length DNA.

The results seen in samples taken from *am3*-infected cells are not unambiguously interpretable. Although the *am3* mutation is associated with a diminished rate of recombination (7, 9),

it is nevertheless possible to obtain at least a small amount of recombinant DNA in this case (21). The structures seen by Tsujimoto and Ogawa (21) were biparental recombinants. Nevertheless, there appeared to be a lower frequency of recombination-related structures in the concatemeric DNA found after infection with *am3* mutants. There may be some difficulty in removing the last traces of host DNA from such samples since the *am3* infection leads to a relatively slow rate of synthesis of T7 DNA and a low amount of concatemeric T7 DNA (Fig. 4). It would be more directly interesting to carry out similar experiments with DNA synthesized in the absence of gene 6, which is generally agreed to be required for recombination in T7 (7, 10, 16). However, it has been shown that in the absence of the gene 6 exonuclease much of the DNA is converted to fragments smaller than unit size (10). The double mutation *am3 am6* does produce some concatemeric DNA, but the amount of host DNA broken down and converted to phage sequences is extremely low. We have found (unpublished data) that only an insignificant amount of the intracellular DNA is in fact present as T7 sequences under these conditions. Therefore, a direct analysis by this technique is not applicable. Powling and Knippers (17) have examined the *am6* mutant DNA by using a density shift approach. They found the density-shifted DNA to contain apparently normal numbers of branches, gaps, and whiskers.

Under the conditions of these experiments (30°C), the conversion of *E. coli* to T7 DNA was complete by about 17 min (Fig. 1). Even after this time, however, there was a rapid incorporation of added [^{3}H]dThd into DNA (data not shown). This was apparently being incorporated into recombining and maturing DNA and did not reflect net T7 DNA synthesis. The incorporated ^{3}H label late in infection is probably entering DNA at a very high specific activity because of a relatively small pool of mononucleotides, host DNA having been degraded and reincorporated. Thus, rates of [^{3}H]dThd incorporation do not reflect the rate of T7 DNA synthesis (17).

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