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Folded, Concatenated Genomes as Replication Intermediates of Bacteriophage T7 DNA

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A complex form of bacteriophage T7 DNA, containing up to several hundred phage equivalents of DNA, arises during replication of T7. The complex was stable to treatment with ionic detergent, Pronase, and phenol. The complex form normally exists for only a short time, corresponding to the phase of rapid T7 DNA synthesis. It is then converted to shorter molecules, both concatemers and unit-size DNA. The complex was stable up to the temperature of denaturation of the bihelix. It consisted of a series of loops emanating from a dense central core, as shown by electron microscopy. The complex form is similar to the relaxed *Escherichia coli* folded chromosome ("nucleoid"). The loops contained an average of 0.7 to 0.8 phage equivalent of DNA. During infection by phage with an amber mutation in gene 3 (endonuclease), formation of the complex occurred normally, but its maturation to unit-size DNA was blocked. Before treatment with phenol, the complex contained short fragments of newly replicated DNA. These were released as single-stranded pieces during phenol treatment. A pathway for T7 DNA replication is indicated in which the flow of material is from unit-size DNA to linear concatemers to the complex form, and then back to unit-size DNA by way of linear concatemers.

Synthesis of host DNA stops, and bacteriophage T7 replication begins, about 6 min after infection of susceptible *Escherichia coli* by T7 (37, 38). During the course of infection, the phage-specific replication system converts all of the host DNA to T7 DNA by way of a rapidly turning-over pool of mononucleotides (37). Replication of parental phage DNA is initiated about 17% from the left genetic end and moves bidirectionally to generate linear, unit-length daughter molecules (9, 40).

The structure of the newly replicated DNA during the subsequent phase of rapid synthesis has been studied a number of times. Pulse-labeled DNA, extensively purified to remove protein, was shown to sediment, in part, faster than unit-length T7 DNA and to contain up to several phage equivalents in a linear molecule (5, 21, 27, 32, 36, 38). T7 DNA molecules of unit length can form concatemers if the terminally redundant sequences become exposed (31). A model for T7 DNA replication, in which such exposure results directly from replication of linear, unit-length molecules, has been proposed. In this model (39) the "sticky ends" so produced lead to the formation of concatemers, which, after replication, regenerate the terminal sequence in becoming mature, unit-length molecules. Some support for such a mechanism is given by the existence of single-stranded gaps at or near "terminal sequences" within concatemers (32).

Genetic evidence also suggests that there is a fast-sedimenting precursor of mature T7 DNA. For example, there is an accumulation or persistence of concatemeric DNA in infected cells when T7 maturation is inhibited by genetic lesions or the drug chloramphenicol (18, 27, 32). Of particular significance is the observation that amber mutations in gene 3, an endonuclease, block genetic and molecular recombination (24, 26, 30) but not the formation of concatemers (16, 27, 36). Thus, concatemer formation does not depend on general recombination.

Several studies have also been made of the structure of replicating T7 DNA not completely freed from possible protein or membrane components. Lysis of T7-infected cells with lysozyme and Triton X-100 yielded a very fast-sedimenting form of T7 DNA, which contained some protein and was largely converted to unit-size DNA by digestion with Pronase (4). More recently, the nonionic detergent Brij-58 was shown to produce a similarly fast-sedimenting structure, which in electron micrographs appeared to consist of up to 30 phage equivalents

of DNA in a complex (33). Subsequent treatment with Pronase or sodium dodecyl sulfate affected these structures only slightly, but they were lost during treatment with phenol. This suggests that the DNA was associated with protein or membrane material and was drawn into the phenol layer thereby (25). It was suggested (33) that the shorter, linear concatemers observed by Schlegel and Thomas (32) may have been degradation products of this larger complex.

The nature of replicating T7 DNA is thus a matter of controversy. Although stable concatemeric structures have been isolated after thorough purification of the DNA, there appear to be larger complexes in the cell that may be unstable to deproteinization and phenol treatment. We have reinvestigated this question and studied the properties and kinetic relationships between fast-sedimenting and other forms of intracellular T7 DNA. Somewhat to our surprise, we found much of the replicating DNA to be in the form of large complexes. These complexes are stable to exhaustive removal of protein and membrane material by a procedure that includes treatment with phenol. Each structure contains most of the intracellular DNA from one cell, at least during the phase of rapid replication. It is converted at later times to mature T7 DNA. Quantitative recoveries and the use of a phage with a gene 3 amber mutation indicate that this structure is on the pathway of T7 DNA replication. Finally, it is possible to rationalize the apparent differences between these observations and the literature quoted above by a careful consideration of the T7 infectious cycle and the procedures for DNA isolation.

MATERIALS AND METHODS

Bacteria, phage, and media. *E. coli* B23 were grown in modified M9 medium as described elsewhere (29). All cultures were maintained at 30°C. Cells at a density between 3×10^8 and 6×10^8/ml were infected with T7 at a multiplicity of 5 to 10. Under these conditions, extensive T7 DNA synthesis began about 9 min after infection and was maximal between 11 and 16 min (cf. reference 21, where maximal synthesis, apparently at 37°C, is between 6 and 12 min). T7 *am*3-29, designated *am*3, was a gift from W. Studier.

Labeling and isolation of intracellular DNA. Infected cells were pulse labeled with 2 to 10 μCi of [^{3}H]deoxythymidine (dThd) (20 to 25 Ci/mmol). Cells were rapidly quenched by pouring them into 1 volume of ice-cold phenol-ethanol killing solution (2% phenol-8 mM EDTA-75% [vol/vol] ethanol-0.02 M sodium acetate buffer, pH 5.3). The work-up was similar to the method described earlier (29); cells were collected by centrifugation, taken up in 50 mM Tris-hydrochloride (pH 8.0)-50 mM NaCl-25 mM EDTA, and treated with 1% Sarkosyl and 2 mg of Pronase per ml for 4 h at 40°C. Proteins and detergent were removed by three phenol extractions, "rolling" samples gently to avoid shear.

Uniform labeling of T7 DNA was achieved by adding 20 μM [^{14}C]dThd (50 mCi/mmol) at 7 min after infection. Uptake of ^{14}C label into T7 DNA was linear up to 18 min postinfection. No increased incorporation was observed when additional [^{14}C]dThd was added between 7 and 18 min, indicating that 20 μM labeled nucleoside saturated the thymidine pools for the duration of the experiment. Thymidine is incorporated almost exclusively into T7 DNA after 7 min, as host DNA synthesis has stopped by this time (21; our unpublished data). By 9 min, at least 90 to 95% of a pulse label was in T7 DNA (unpublished data).

Double labeling of T7 DNA was used to study both the bulk of intracellular T7 DNA (^{14}C) and pulse-labeled, newly replicated structures (^{3}H). A large culture was infected and divided. One portion was then uniformly labeled with [^{14}C]dThd from 7 min, as described above. Aliquots (3 ml) of the other portion were pulse labeled (15 s) with [^{3}H]dThd at the times indicated and quenched. Within 5 s, 3 ml of the ^{14}C-labeled culture was poured into the same quenching solution. This protocol led to the incorporation of reasonable ^{3}H label in a short pulse, without having to resort to excess levels of [^{3}H]dThd, as would have been the case if it were added directly to the [^{14}C]dThd-containing cultures.

Characterization methods. Neutral sucrose gradients (5 to 20%) were made in 1 M NaCl-0.05 M Tris-hydrochloride (pH 7.5)-5 mM EDTA. They contained a bottom shelf of saturated Cs_2SO_4 in 20% sucrose. Alkaline sucrose gradients were made in 0.9 M NaCl-0.1 M NaOH-5 mM EDTA, with no shelf. Samples for alkaline sedimentation were exposed to 0.3 M NaOH for 1.5 h at room temperature before being layered onto the gradients. Centrifugation was in a Beckman SW50.1 rotor, at 4°C, in all cases. Fractions were pumped out directly onto filter-paper disks, which were then washed in trichloroacetic acid and assayed for radioactivity.

Analysis on agarose columns and hydroxyapatite (28, 29) and preparation of unit-length ^{32}P-labeled phage DNA (27) have been described.

Fast-sedimenting DNA complexes were isolated by sedimenting them to the bottom of a sucrose gradient without a shelf (30,000 rpm for 2 h at 5°C). The pellet was gently resuspended in a small volume of 0.05 M Tris-hydrochloride (pH 8.0)-1.0 M NaCl-5 mM EDTA and then diluted with the same buffer containing 0.1 mg of bovine serum albumin per ml. The DNA was pelleted again, this time by centrifugation for 30 min at 30,000 rpm and 15°C.

Electron microscopy. Samples were prepared for electron microscopy by the spreading technique described by Davis et al. (7). The DNA complexes were diluted to a concentration of approximately 0.5 μg of DNA per ml in a buffer containing 0.1 M Tris-hydrochloride (pH 7.5), 0.01 M EDTA, 40% formamide (Matheson, Coleman and Bell), and 0.01% cytochrome *c* (Sigma type V). A 50-μl amount of this

solution was spread onto a hypophase containing 0.01 M Tris-hydrochloride (pH 7.5), 0.001 M EDTA, and 10% formamide. The DNA-protein film was mounted on Parlodion-coated copper grids, stained with uranyl acetate, and shadowed with Pt/C in a Balzers BA-511 M apparatus.

Micrographs were taken with a Philips EM 300 electron microscope operated at 60 kV, using a 30-μm objective aperture. The instrumental magnification was ×5,000 (calibrated with a diffraction grating replica).

RESULTS

Sedimentation of uniformly and pulse-labeled intracellular T7 DNA. The progeny T7 DNA in infected *E. coli* cells was uniformly labeled with ^{14}C and pulse labeled with ^{3}H for 15 s at various times after infection. The cells were quenched, and the DNA was isolated using Sarkosyl, Pronase, and phenol. The recovery of incorporated pulse label between 9 and 15 min was, on average, 81% at the postphenol stage. Uniform, bulk T7 label recovery averaged 73% at this stage. Bulk label at late times (after 15 min postinfection) was recovered completely (average, 101%). Recovery of labeled DNA added to sucrose gradients averaged better than 90% for both pulse- and bulk-labeled material.

Analysis of the sedimentation pattern between 9 and 21 min is shown in Fig. 1. At early times, the bulk T7 DNA (^{14}C label) was largely in the unit- to short-concatemer-length region, but some of it was fast sedimenting. By 13 min, the major part was fast sedimenting. Beginning at 15 min, and becoming particularly evident by 18 min, there was a sharply defined, unit-sized population, corresponding to mature T7 DNA. By 21 min, essentially all T7 DNA was in this class. (Lysis occurred between 29 and 31 min under these conditions.)

The size of the putative unit-size DNA was confirmed by centrifugation against [^{32}P]DNA isolated from phage particles. It was also shown to consist primarily of unit-size chains by centrifugation in alkaline gradients (data not shown).

Pulse-labeled DNA (15 s of [^{3}H]dThd) exhibited the same general patterns as uniformly labeled DNA, but with some differences. At 9 min the pulse label appeared preferentially in moderately long concatemers, relative to fast-sedimenting complexes. By 13 min, it was preferentially in the complexes, and at 18 and 21 min it was incorporated into shorter concatemers.

Fast-sedimenting complexes contain essentially only T7 DNA sequences. This was determined by first uniformly labeling the DNA of uninfected *E. coli* with [^{14}C]dThd. At 14.5 min after subsequent infection with T7, [^{3}H]dThd was added, and at 15 min the cells were quenched. Fast-sedimenting complexes were isolated as described above, heat-denatured in the presence of authentic, unlabeled T7 phage DNA, and allowed to anneal. Under the conditions used, 90% of the ^{3}H and 81% of the ^{14}C annealed. (*E. coli* DNA was shown not to anneal under the conditions used; see 28.) Since the host DNA was uniformly labeled before infection, this result demonstrates the relative absence of *E. coli* DNA in the fast-sedimenting complex (<10%). The ^{14}C label found in fast-sedimenting T7 DNA has been re-incorporated after hydrolysis of the *E. coli* genome (37).

Several other control experiments were also performed. DNA from uninfected *E. coli*, uniformly and pulse labeled as above, did not produce the very fast-sedimenting structures seen after T7 infection. The average sedimentation coefficient of *E. coli* DNA, isolated as above, was 69*S*. This corresponds to native DNA of molecular weight about 230×10^6 (approximately at the position marked "10×" in Fig. 1). DNA isolated from uninfected *E. coli* cells did not show any of the complex structures described below when examined by electron microscopy. Fast-sedimenting DNA from pulse-labeled, T7-infected cells resediments true, to the extent of 70%; thus the structures are fairly stable. Finally, the distribution of radioactive material was identical when a split sample was analyzed either by puncturing the bottom of the centrifuge tube and pumping out the gradient (the usual method) or by displacing the sample up through the top of the tube with a dense solvent. Thus, the position of the very fast-sedimenting material was not an artifact of the method used to analyze the gradients.

Electron microscopy of fast-sedimenting complexes. Samples of fast-sedimenting DNA, prepared as described above (phenol treatment included), were isolated and examined by electron microscopy (Fig. 2 and 3). These contained large, complex DNA structures, with double-stranded loops emanating from a dense central core. Although short pieces of DNA were also found on the grids, by far the major portion was in complex structures.

The micrograph of the complex shown in Fig. 2 was projected and traced on a large sheet of paper. The total amount of DNA in the complex was estimated with a map measurer, assuming that all strands entering the central dense area extended through it. The total content was estimated to be 114 phage equivalents of DNA. The number of strands crossing a circle drawn around the core was 300 ± 10. This number,

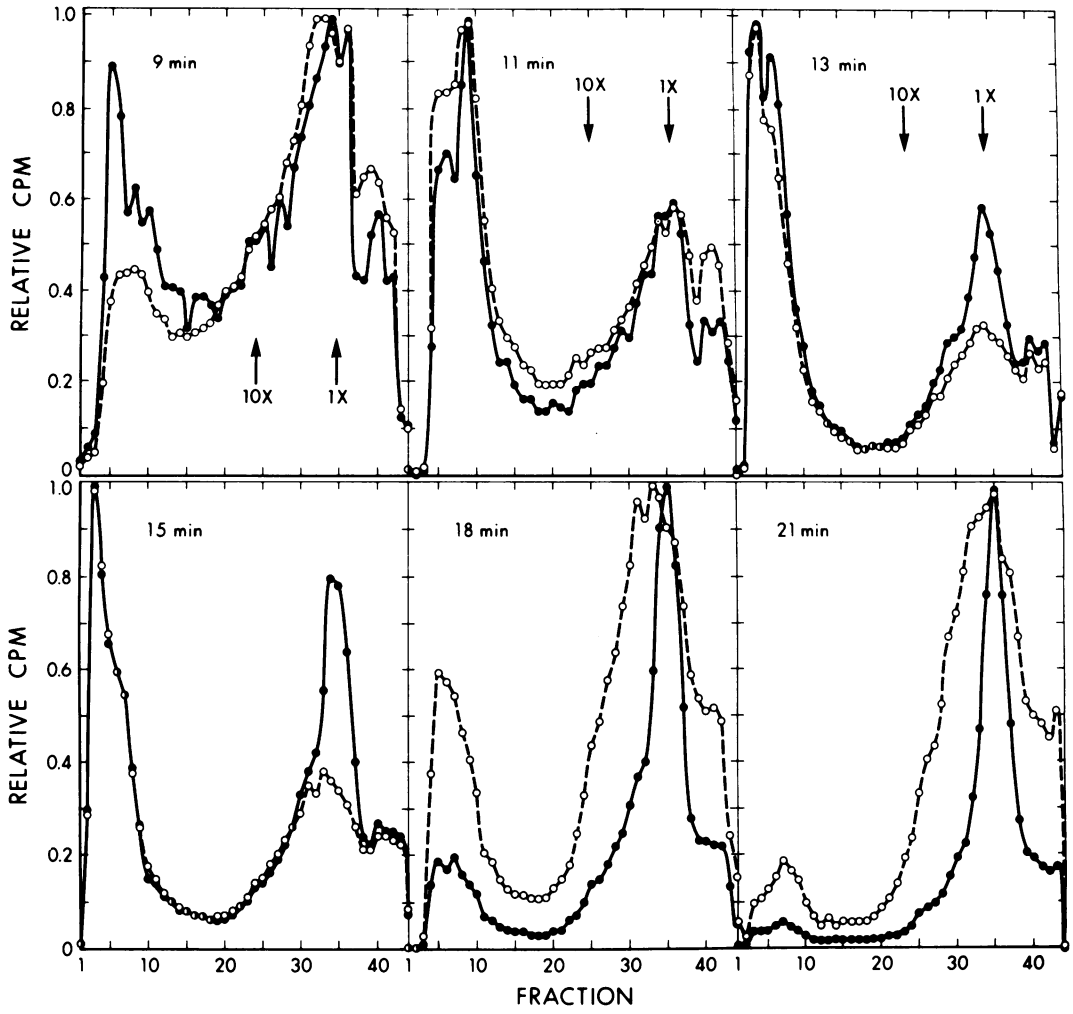


FIG. 1. *Sedimentation of intracellular T7⁺ DNA. E. coli infected with wild-type T7 phage were labeled with ^{14}C (uniform T7 label, ●) and ^{3}H (15-s pulse, T7 label, ○) at the times after infection indicated. Conditions are described in the text. The DNA isolated using Sarkosyl, Pronase, and phenol was layered onto 5 to 20% sucrose gradients and centrifuged for 2 h at 30,000 rpm and 5°C in an SW50.1 rotor. The calculated positions of DNA 10 phage equivalents long is indicated as "10×." The unit-sized DNA ("1×") was also examined in gradients at 45,000 rpm and co-sedimented with ^{32}P-labeled DNA isolated from phage. Sedimentation is from right to left. Radioactivity is expressed relative to the peak fraction for each isotope, and recoveries are discussed in the text. The 9-min sample represents DNA from 2.4 ml of infected cells; all the other samples represent 1.2 ml. The total ^{14}C (uniform) T7 label in these samples before centrifugation was: 9 min, 6,070 cpm; 11 min, 8,250 cpm; 13 min, 12,000 cpm; 15 min, 17,580 cpm; 18 min, 21,760 cpm; 21 min, 25,035 cpm. The total ^{3}H (pulse) label was: 57,600, 71,560, 94,500, 92,300, 57,100, and 24,700 cpm at the same times, respectively.*

somewhat surprisingly, was invariant when the enclosing surface was moved out to various distances, up to about 2/3 the radius of the complex. (The "edge" of the complex was taken to be the distance at which the DNA content per concentric shell fell off, which it did, quite abruptly.) Thus, the average loop contained approximately 0.76 phage equivalent by this calculation. Seven isolated loops were measured directly, on this and other complexes, and yielded an average length of 0.71 phage equivalent of DNA. A series of complexes yielded 125 to 250 loops (average, 196 loops), suggesting a total DNA content of about 145 phage equivalents. At this time after infection (15 min), the average cellular content of T7 DNA was about 200 phage equivalents (data not shown).

Release of nascent, single-stranded DNA by phenol. Newly replicated T7 DNA releases nascent, single-stranded fragments during treat-

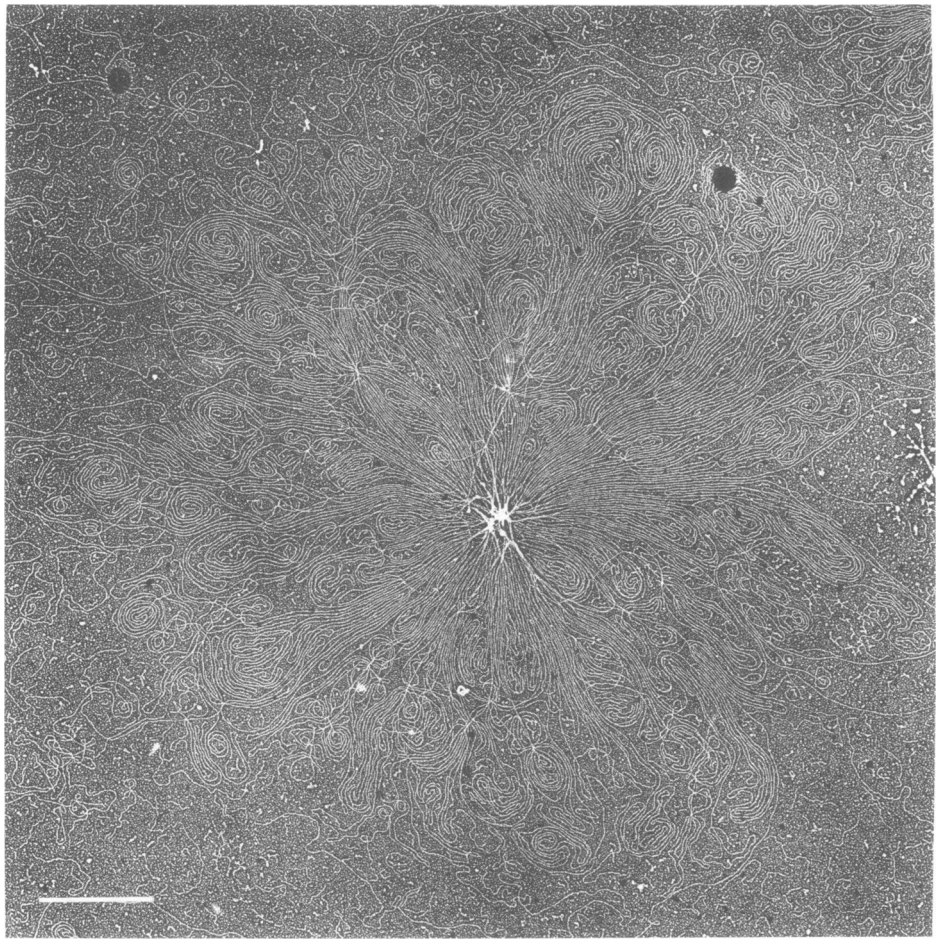


FIG. 2. *Electron micrograph of T7⁺ fast-sedimenting DNA (15 min after infection). After uranyl acetate staining, this DNA was shadowed, while rotating, with 2 nm of Pt/C. × 15,000 (bar, 1 μm). The micrograph has been printed in reverse contrast.*

ment with phenol (29). We observed that phenol-treated samples contained a slowly sedimenting component, which was not present before phenol treatment (Fig. 1). As will be shown, this slowly sedimenting component contained nascent, single-stranded fragments. Another effect of phenol treatment was to reduce the amount of fast-sedimenting DNA. This was particularly noticeable at early times and is illustrated for a 10-s pulse-labeled sample 9 min after infection (Fig. 4). Before phenol treatment, but not after, a significant part of the label had a high sedimentation rate. The phenol-treated sample contained a component sedimenting slower than unit size. The same pattern of results was seen at later times, except that there was an increasing fraction of T7 DNA in the phenol-stable, fast-sedimenting complex.

The material sedimenting slower than unit size from a pulse-labeled, 13-min sample was found to contain 38% single-stranded DNA by hydroxyapatite analysis, using methodology we have already described (28, 29). In a more direct test of the effect of phenol, fast-sedimenting, non-phenol-treated DNA was isolated from cells labeled for 5 s at 13 min after T7 infection. This material released 12.9% of the radioactive label as short, single-stranded fragments upon treatment with phenol. On the other hand, reextraction with phenol of fast-sedimenting complexes prepared as in Fig. 1 (i.e., using phenol in the work-up) did not release single-stranded fragments. The lesser amount of fast-sedimenting complex in this experiment relative to the 9-min sample in Fig. 1 is due partly to the shorter pulse time. More label runs with the slowly sedimenting, single-stranded nascent

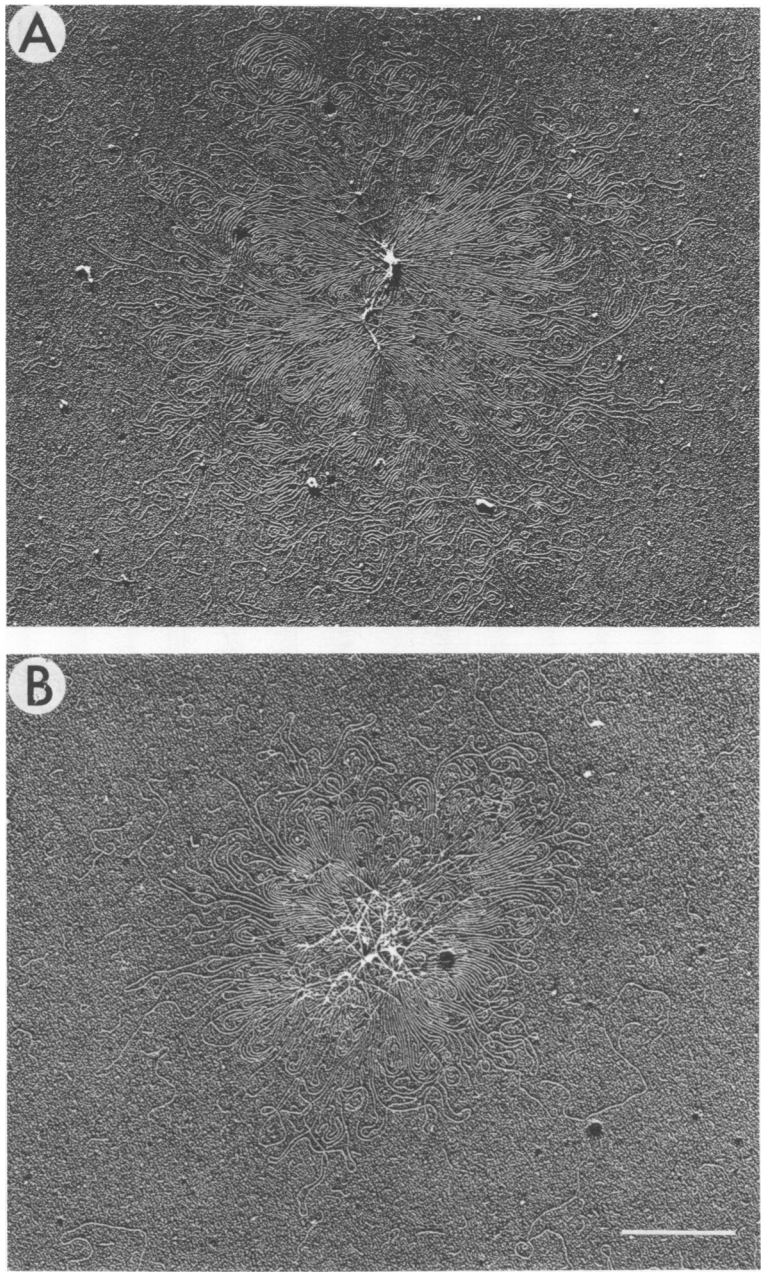


FIG. 3. *Representative fast-sedimenting complexes of T7⁺ (A) and T7 am3 (B) DNAs obtained from parallel cultures (15 min after infection). These samples were stained with uranyl acetate, rotary shadowed with 1 nm of Pt/C at an angle of 10°, and then shadowed at an angle of 5° with an additional 1.5 nm of Pt/C. ×15,000 (bar, 1 μm). Printed in reverse contrast.*

fragments in the sample pulsed for a shorter time. Part of the variation may also be due to experimental differences.

In summary, another type of very fast-sedimenting complex, which forms earlier during infection and is phenol sensitive, can also be demonstrated. Upon phenol extraction, it releases nascent, single-stranded DNA fragments. At least at early times after infection, the complex then reverts to sedimentation behavior characteristic of moderately long concatemers (Fig. 1).

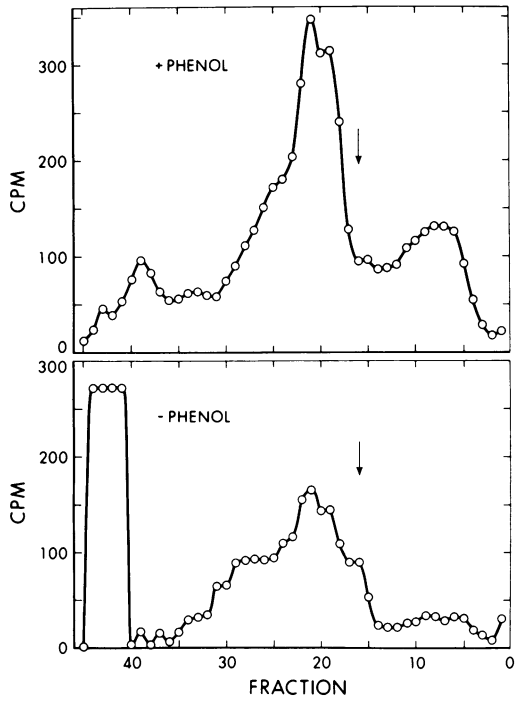


FIG. 4. *Existence of a phenol-sensitive, fast-sedimenting complex early in infection. T7 DNA was pulse labeled for 10 s at 9 min after infection. After the cells were quenched, they were divided, one portion being purified by treatment with Sarkosyl and Pronase (−phenol), and the other being subjected to phenol extraction as well (+phenol). Sedimentation (45,000 rpm for 2 h at 5°C in an SW50.1 rotor) is from right to left. The position of ^{14}C-labeled DNA isolated from T7 phage is indicated by the arrow. The fast-sedimenting component of the −phenol sample actually was located in a sharp peak at the interface of the gradient and supporting shelf (see text), but has been leveled off to make representation easier. The total counts in this component are correct as represented.*

Stability of the fast-sedimenting DNA complex. The fast-sedimenting DNA complex isolated on sucrose gradients (after phenol extraction) was found to be relatively stable to heating (Fig. 5). At very low ionic strength (0.025 M NaCl), about half the complex was disrupted by heating to 80°C, as measured by its sedimentation properties. The DNA duplex was somewhat more stable, having a melting temperature of about 85°C. Increasing the salt concentration, or adding ethidium, stabilized both the complex and the DNA duplex. Thus, the fast-sedimenting complex has the stability characteristics expected of a structure maintained by DNA duplex interactions. The sedimentation behavior of the complex was unaffected by treatment with 20 μg of RNase A per ml for 30 min at room temperature.

Complexes from *am*3 (endonuclease-deficient) T7-infected cells. Mutants in T7 gene 3 are unable to carry out genetic or molecular recombination, but they do undergo at least a limited amount of replicative DNA synthesis. Gene 3 mutants produce DNA complexes that sediment rapidly in neutral sucrose gradients (see above). The results of analyzing uniformly and pulse-labeled DNA from cells infected with T7 *am*3-29 are shown in Fig. 6. The data differ from those of wild-type T7 DNA (Fig. 1) in two ways. At early times, *am*-3 processes lagged behind those of T7$^+$. Thus, at 9 min, none of the *am*-3 DNA was fast sedimenting. By 15 min, the patterns of *am*-3 and T7$^+$ were essentially the same as far as bulk T7 DNA was concerned.

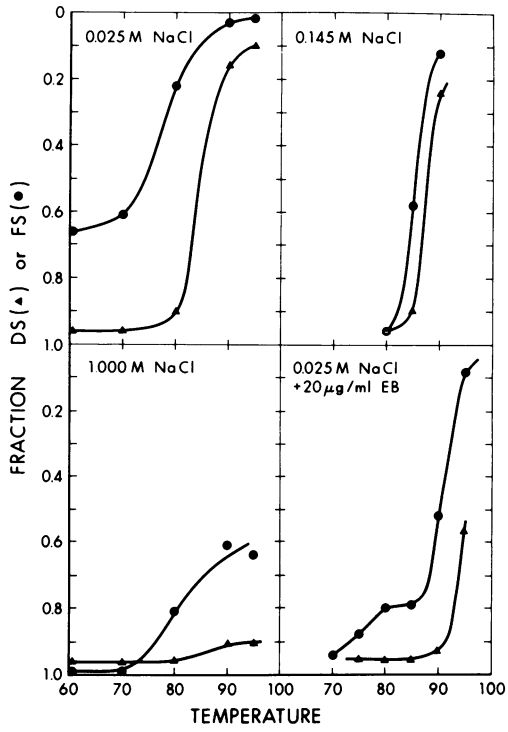


FIG. 5. *Stability of isolated fast-sedimenting complexes to heating. T7 DNA was pulse labeled (60 s) 15 min after infection, and fast-sedimenting complexes were isolated after complete deproteinization, including phenol treatment (see text). The resuspended pellet was then heated to various temperatures, and samples were taken for analysis of fast-sedimenting (FS) forms (by centrifugation) and double strandedness (DS) (by hydroxyapatite). The amount of fast-sedimenting material is expressed relative to that in unheated samples, which varied between 50 and 64% in these experiments. Heating was done by incubating samples at the various salt concentrations or with ethidium bromide (EB) and slowly (over a period of about 50 min) raising the temperature to 95°C. At appropriate temperatures, samples were removed for analysis.*

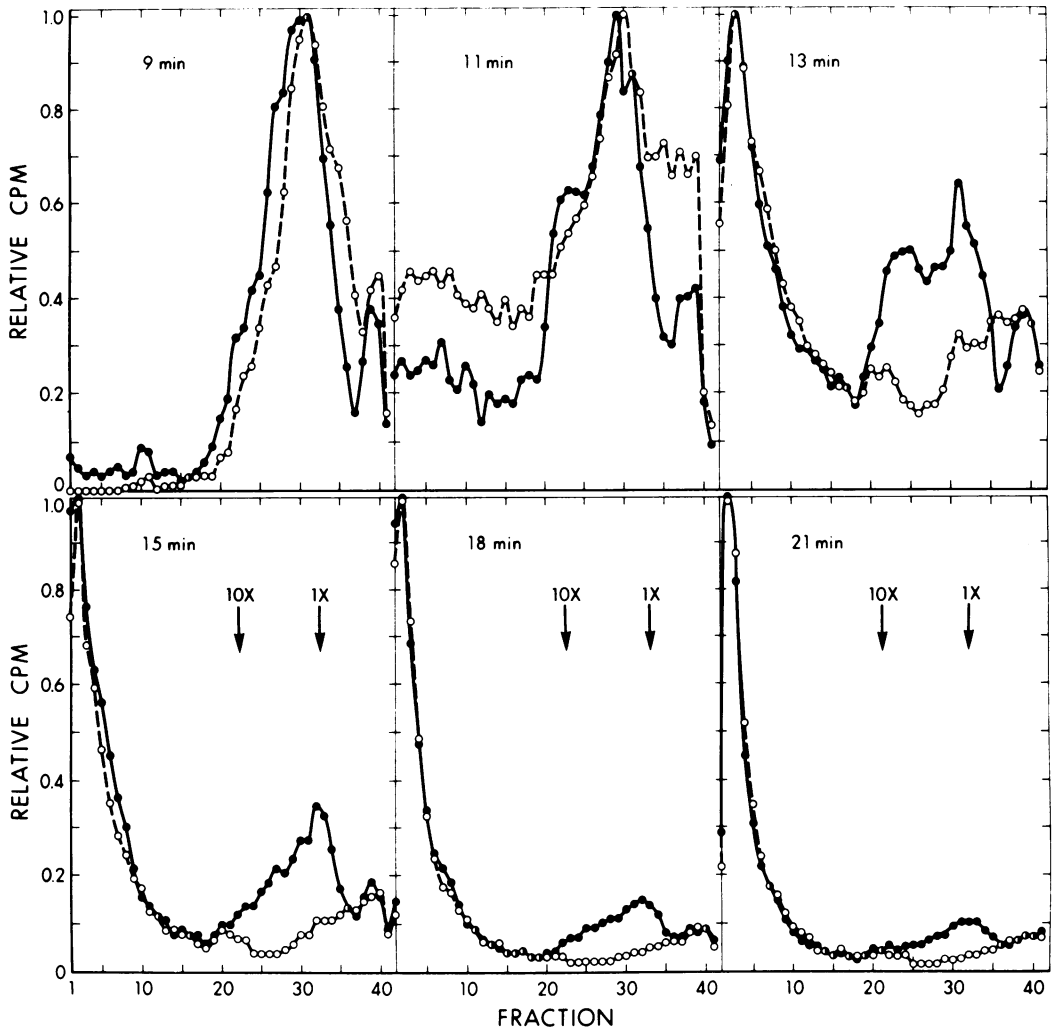


FIG. 6. *Sedimentation of intracellular T7 am3-29 DNA. The conditions of this experiment, performed using a T7 strain defective in gene 3 (endonuclease) are identical to those of Fig. 1.*

The second difference became apparent by 18 min after infection. By this time, T7$^+$ DNA began to mature to unit size, and a pulse label was incorporated into both concatemers and fast-sedimenting forms. In the *am3* case, there was no evidence for this maturation step. This difference was even more apparent by 21 min. Essentially all T7$^+$ DNA was now of unit size, and none was fast sedimenting. In *am3*, the reverse was true.

Electron microscopy of fast-sedimenting DNA from *am3*-infected cells showed the complexes to be similar to those of wild-type DNA. However, the *am3* complexes were distinguishable from those of T7$^+$ by virtue of their more diffuse central regions (Fig. 3). In addition, although quantitative comparisons have not been made, the *am3* complexes appeared to contain less DNA than those of T7$^+$ when isolations were made from parallel cultures.

DISCUSSION

At least some of the newly synthesized DNA isolated from T7-infected *E. coli* during the rapid phase of DNA replication has been shown to be in the form of compact, high-molecular-weight structures. These structures consist of double-stranded loops, about 0.75 phage equivalent long, emanating from a dense central core. There is no evidence for other macromolecules being important to the stability of the isolated complexes, since they are stable to ionic detergents, Pronase, and phenol. Also, we have seen no evidence for associated "debris" in electron micrographs, or of an anomolous density in CsCl gradients (unpublished data).

Similar structures have been found for replicating T4 phage DNA (6, 12–14, 17, 20, 23) and herpesvirus DNA (1). The complexes seen in electron micrographs were not shown to contain viral DNA directly, but labeled DNA known to be herpesvirus sequences did have the sedimentation rate characteristic of fast-sedimenting complex. The various complexes are compared in Table 1. Each is present after exhaustive purification and consists of compact structures, dominated by loops emanating from a central core. These viruses share the feature of having terminally repeated sequences, which may be significant. Several less-well-characterized complex forms have also been described (see Introduction). In the case of T7, these generally have been the product of incomplete removal of protein and membrane material (2, 4, 33). The fast-sedimenting form of DNA observed before phenol treatment (Fig. 4) is probably similar to these incompletely purified complexes seen earlier. However, several authors have observed very fast-sedimenting, transient forms of replicating T7 DNA that are stable to extensive purification (5, 32, 35, 36). These fast-sedimenting forms are probably the same as the ones described in this paper. Stabilization of fast-sedimenting DNA structures by a lesion in gene 3 has also been shown (36). Concatemeric T7 DNA (up to 10 phage equivalents long) has been shown to be stabilized by mutations in genes 8, 9, 10, 18, 19 (32), 3, and 6 (27, 36). Concatemers are apparently not formed when gene 2 is malfunctional (5). If, as we propose here, the complex form is a precursor to mature, unit-length phage DNA, it is not surprising that mutations both in structural proteins and the gene 3 endonuclease interfere with its metabolism. The reasons that the complexes might not always appear to be quantitatively important in wild-type infections include (i) their rather short lifespan, even at 30°C, as shown here, and (ii) the sometimes overlooked importance of thorough digestion with Pronase to insure the quantitative recovery of replicating structures after a subsequent phenol treatment (25).

Do the complex forms of replicating T7 DNA exist in the cell, or are they artifacts of DNA isolation? Evidence that such structures exist intracellularly includes the following. (i) They have been isolated from a variety of virus-infected cells (Table 1) by various workers. (ii) A high concentration of T7 DNA, either intracellular or in the lysed sample, does not by itself produce them, because late in infection all the DNA sediments as unit-length duplexes. (iii) The complexes appear to be organized, as already described, around a central core, and in the case of T4 complexes replication appears to be core associated (20). (iv) Complexes contain an amount of DNA similar to the intracellular content of phage DNA, in the cases of T7 (our data) and T4 (20). If complexes were formed non-specifically after lysis, they might just as easily be much smaller or much larger than this. (v) The absence of a function (gene 3 endonuclease) known to act on single-stranded regions of DNA is associated with the persistence of fast-sedimenting structures. It therefore seems

TABLE 1. *Complex forms of replicating viral DNAs*

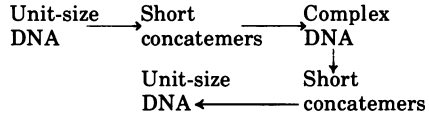
Virus	Genome[a]	Isolation method[b]	Properties of complex form
Herpes	MW = 100 × 10^6	ID, Pronase, phenol (1)	S = 70–150.
			"Loops" and "centers"
	Terminal repeat		Mass: up to 0.25 × 10^9 daltons
			Free of RNA, protein, membranes
			Buoyant density normal for DNA
T4	MW = 106 × 10^6	Lysozyme, ID ± Pronase (12, 20)	$S \geq 200$ (12) in wild type
	Terminal repeat, permuted sequences		$S > 1{,}000$ in gene 49^- mutants (15)
			"Loops" and "centers" (20, 22)
			Average loop, 10 μm
			Mass: up to 10 × 10^9 daltons
			Free of protein
T7	MW = 25.2 × 10^6	ID, Pronase, phenol	S = 440
			"Loops" and "centers"
	Terminal repeat		Average loop, 9.5 μm
			Mass: up to 5 × 10^9 daltons
			Free of protein, RNA, membranes
			Buoyant density normal for DNA (this paper)

[a] MW, Molecular weight.
[b] ID, Ionic detergents, Sarkosyl or sodium dodecyl sulfate.

likely that the function of the gene 3 endonuclease is normally to convert fast-sedimenting complexes to shorter concatemers or unit-length molecules.

Although some sort of complex form does appear to exist, its exact nature before cell lysis cannot be defined. The central core, for example, might result from coalescence of single-stranded regions during lysis and removal of protein. In the present work, as in the case of T4 (12), the complexes are quite stable. The T7 complex dissociates at the melting temperature of duplex DNA, and this is true even when the melting temperature is increased by adding salt or ethidium bromide. Addition of ethidium bromide does not produce supercoiled complex DNA (unpublished data), but whether closed loops existed in the cell is not disproved thereby. Treatment of the T7 complex with phenol releases nascent, single-stranded fragments (29), perhaps by branch migration. Prephenol complexes differed little from the electron micrographs shown here, except for the presence of more debris.

Replication of T7 begins on unit-length parental DNA (9). Concatemeric-sized intermediates, up to about 10 phage equivalents, become labeled early in infection. This is followed by labeling of the fast-sedimenting complex form. Pulse-labeled DNA at late times preferentially enters shorter concatemers again (Fig. 1) in T7$^+$-infected cells, during a time when maturation to unit-length DNA is occurring. The conversion of bulk T7 DNA to unit length and the late-time pulse labeling of shorter concatemers are blocked in the case of T7 *am*3. The pathway of DNA replication and maturation suggested by these data is:



The final step has been shown to involve unit-sized DNA with single-stranded regions as an intermediate (32). Repair of these regions plus recombination-associated repair could account for the substantial labeling of short concatemers. The mechanism for excision of unit-length, mature DNA from concatemeric intermediates proposed by Watson (39) would also involve DNA synthesis.

The advantages to the phage of a compact replicative form would presumably include having the appropriate substrates in a concentrated form and having the enzymatic machinery highly organized. At its peak rate, T7 DNA synthesis is about seven times as fast as *E. coli* DNA replication on a per-cell basis.

The existence of compact, folded genomes may be a fairly general phenomenon. The most intensively studied structure of this type is the *E. coli* nucleoid (34). The intact nucleoid consists of about 50 loops of DNA, apparently held together by RNA (41). The DNA loops are closed, and supercoiling of these gives the nucleoid a very high sedimentation coefficient. When the DNA in each loop is nicked by DNase, the resulting relaxed complex has a sedimentation coefficient about 60% as high. Earlier reports measured these sedimentation coefficients relative to that of bacteriophage T4 particles, which were assumed to have a value of 1,025*S*. The correct value is actually 890*S* (11). Our own results also agree with this value. With this correction, and a slight correction for the different partial specific volumes of T4 phage ($\bar{v}$ = 0.617 [11]) and sodium DNA ($\bar{v}$ = 0.556 [19]), the sedimentation coefficient for the unit-sized *E. coli* chromosome in the relaxed, folded state is 660*S*. Its molecular weight is 2.5 × 10^9 (3). This leads to a calculated diffusion coefficient, *D*, of 1.45 × 10^{-9} cm^2/s. The frictional coefficient of this structure relative to that of an unhydrated sphere, *f*/*fo*, is given by 1.01 × 10^{-8} $[(1 - \bar{v}\rho)/D^2 S\bar{v}]^{1/3}$ and is equal to 18. For the T7 complex isolated from *am* 3-infected cells, the molecular weight was estimated as 1.6 × 10^9 by comparing electron micrographs and DNA synthesis rates with wild-type T7. This DNA was examined by centrifugation in sucrose gradients (15,000 rpm for 60 min), using T4 and T7 phage (*s* = 890 and 453*S*, respectively; 11) as markers. The *s* value was 440*S*. This value is only an approximation, as the profile indicated heterogeneity in sedimentation. The effects of intra- and intermolecular interactions on the *s* value have not been estimated. We think that these interactions will have an insignificant effect on *s* because of the low rotor speed used and the compactness of the fast-sedimenting complexes. Assuming that $\bar{v}$ = 0.556, one can calculate that *D* = 1.5 × 10^{-9} cm^2/s and *f*/*fo* = 18. Thus, the asymmetry and hydration properties of the T7 folded, concatemeric genome described here are similar to those of the relaxed folded *E. coli* chromosome. The high value of *f*/*fo* for such structures, which by electron microscopy appear to be relatively symmetrical, is puzzling. Whereas the extremely large *f*/*fo* value for normal DNA of this size (about 80, assuming *S* = 150 for the intact *E. coli* chromosome; 10) might be ascribed to its rigid, asymmetric structure, other considerations, such as the effect of hydrodynamically

bound solvent, must apparently be made for the compact, folded genomes. The similarity between folded concatemeric genomes of T4 (20, 22), T7 (this paper), herpesvirus (1), and the *E. coli* nucleoid (8) includes their similarity in electron microscopic observations. Where the *E. coli* nucleoid differs markedly from the T7 complex, at least, is in its sensitivity to sodium dodecyl sulfate or RNase (41), agents that do not affect the T7 structures. The surprising stability of T7 DNA complexes to mechanical shearing is undoubtedly related to their compact structure.

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