Some Properties of DNA from Phage-Infected Bacteria

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ABSTRACT Replicating T5 or λ phage DNA has been labeled by adding tritiated thymidine for short periods to cultures of phage-infected *Escherichia coli* before isolation of intracellular DNA. Two procedures are described for separating T5 replicating DNA from DNA of intracellular phage particles. Both T5 and λ replicating DNA had the same bouyant density in cesium chloride as DNA from phage particles but sedimented faster when centrifuged in sucrose density gradients. The fast sedimentation did not appear to be caused by DNA protein or DNA-RNA complexes or by aggregation of DNA, but is probably due to DNA molecules of unusual structure. Experiments involving hydrodynamic shear and sucrose density gradient centrifugation at alkaline pH have suggested that with λ the replicating form of DNA is a linear molecule considerably longer than the DNA molecules of λ-phage particles. The constituent polynucleotide chains of λ but not T5 replicating DNA also appear to be longer than those of phage DNA.

The replicating chromosome of *Escherichia coli* is circular with normally one growing point (1). Since little is known about the structure of replicating phage DNA, we have isolated DNA from phage-infected bacteria and examined some of its physical properties.

Phage-infected bacteria contain phage-specific DNA of at least two kinds, namely, DNA of intracellular phage particles (condensed DNA) and DNA of the precursor pool, some or all of which is replicating. By subtracting the amount of DNA in infective intracellular phage from the total phage DNA (recognizable by the content of hydroxymethyl cytosine), Hershey and coworkers measured the size of the precursor pool in T2-infected bacteria (2, 3). They showed that the pool of precursor DNA increases until it reaches a level of 30 to 40 phage DNA equivalents per bacterium at 12 min after infection. After this time there is a linear accumulation of DNA in completed phage particles, and the DNA content of the precursor pool remains constant. Electron microscopy of thin sections of T2-infected bacteria revealed the pool of precursor DNA as a fibrillar mass and showed the accumulation, from 10

min after infection, of dense particles similar in shape to phage heads (4). Information about the pools of DNA in bacteria infected with other phages is not as complete.

We have studied intracellular DNA from *Escherichia coli* infected with T5 and λb^+c_1. These two phages were chosen because two different circular forms of λ-DNA have been found (5–7) whereas with T5 DNA no evidence of circularity has been obtained from either physical or genetical experiments. Phage T5 offers the added experimental advantage that the bacterial DNA in T5-infected cells is rapidly degraded after infection (8, 9).

In a typical experiment, a culture of infected bacteria was given a short exposure to tritiated thymidine, immediately chilled, and lysed with lysozyme and detergent. After extraction with phenol the lysate usually contained 90% of the labeled DNA and less than 0.4% of the original protein. Only DNA replicating during the interval of the "pulse" becomes labeled in this type of experiment. For analytical purposes it was compared with the DNA of purified phage particles. In other experiments the bacteria were infected with ^{32}P-labeled phage and then pulsed with tritiated thymidine, so that the parental DNA could be recognized and compared with the newly synthesized DNA in the same culture.

PROPERTIES OF PULSE-LABELED DNA FROM PHAGE-INFECTED BACTERIA

Buoyant Density in CsCl Pulse-labeled DNA from bacteria infected with either phage had the same buoyant density in cesium chloride as DNA from the respective phage particles (Fig. 1). Satellite bands were not observed. With T5 pulse-labeled DNA, even at pH 11.0 where single-stranded DNA has a considerably higher density (10), no differences in buoyant density were detected. These results show that the pulse-labeled DNA was mainly double-stranded and was not attached to measurable amounts of protein.

Column Chromatography of Pulse-Labeled DNA When DNA from pulse-labeled T5-infected bacteria was passed through a column of kieselguhr coated with methylated serum albumin, two major components were resolved (Fig. 2) (11). One (peak 1) was eluted at the salt concentration required to elute DNA from phage T5, and was of low specific activity (counts/micromole deoxyribose). The other (peak 3) was of high specific activity and could be eluted only with ammonium hydroxide. The DNA of the first component, except in the leading edge of the band, had the same sedimentation coefficient as normal T5 DNA. Pulse-chase experiments showed that isotope moved from the peak-3 component to the peak-1 component. Therefore peak 3 contains a replicating form of DNA that is precursor to peak 1, which must be DNA from completed or nearly completed phage particles.

The firm binding of pulse-labeled DNA to methylated albumin columns is

characteristic but unexplained. It could reflect short, single-stranded regions in the DNA or other structural peculiarities associated with replication. Similar behavior of DNA from T4-infected bacteria has been observed by others (12).

Concentration of Pulse-Labeled DNA at the Interface during Phenol Extraction

When a culture of T5-infected bacteria, pulse-labeled at 35 min after infec-

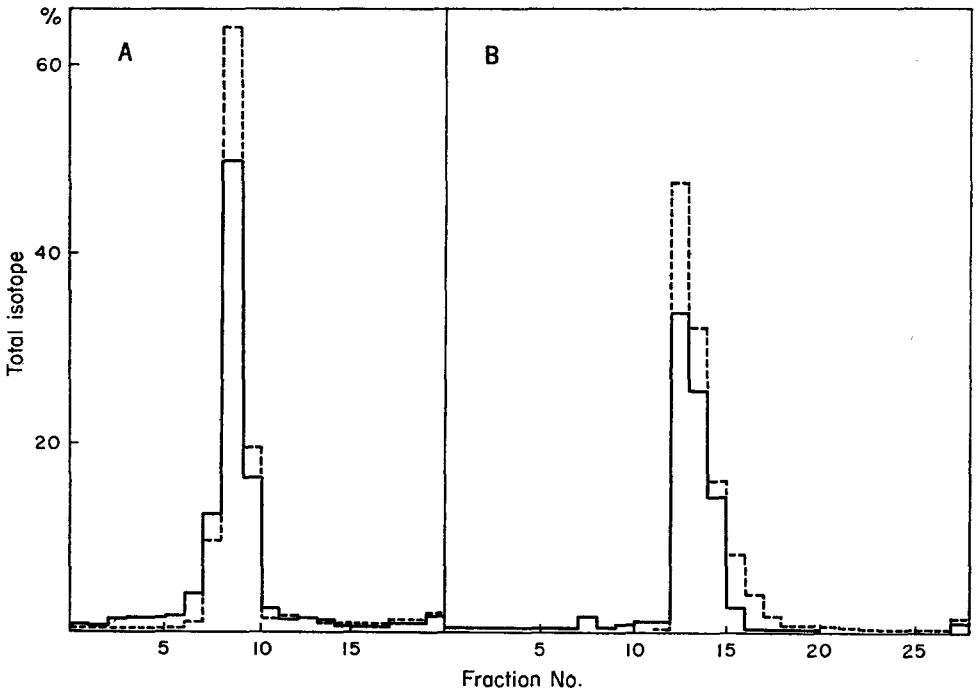

FIGURE 1. Pulse-labeled DNA from (*A*) λ-infected *E. coli* and (*B*) T5-infected *E. coli* in a density gradient of cesium chloride. A mixture of ^{3}H–pulse-labeled DNA and the appropriate ^{32}P-labeled phage DNA (total DNA per tube 5 μg) was centrifuged in cesium chloride solution (ρ = 1.7) for 48 hr in a preparative ultracentrifuge. Fractions were obtained by piercing the tube and collecting drops directly into counting vials. Solid line, ^{3}H; broken line, ^{32}P.

tion, was treated with detergent and phenol without lysozyme digestion, only about 50% of the DNA was found in the aqueous phase (Table I). The remainder was found in the precipitate at the interface between the water and the phenol. The interface DNA contained some 90% of the isotope, and pulse-chase experiments showed that it was a precursor of the DNA of the aqueous phase (11). The aqueous phase DNA chromatographed on kieselguhr columns and sedimented in sucrose density gradients like DNA extracted from phage particles, and must have been derived from complete or nearly complete intracellular phage particles.

A similar kind of fractionation with phenol was found for T2-infected bac-

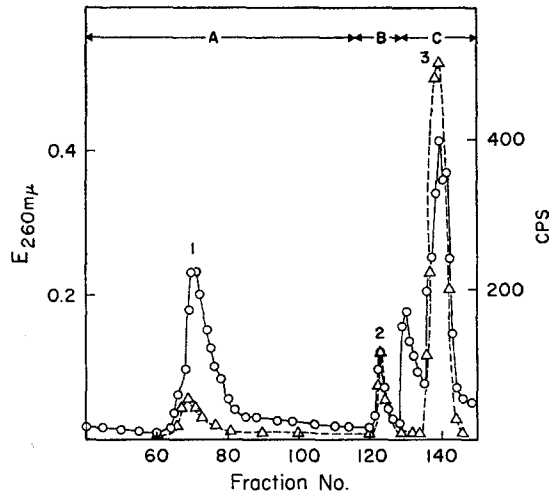

FIGURE 2. Column chromatography of pulse-labeled DNA from T5-infected *E. coli*. A culture of T5-infected *E. coli* was labeled with ^{3}H-thymidine for 1 min at 33 min after infection. The DNA was isolated, and after treatment with ribonuclease a sample (1.1 mg) was applied to a column of kieselguhr coated with methylated serum albumin. The column was successively washed with (*A*) a linear gradient from 0.65 to 0.85 M NaCl containing 0.05 M phosphate buffer pH 6.7, (*B*) 1.5 M NaCl buffered at pH 8.0 with 0.1 M Tris, (*C*) 1.5 M NaCl containing 2 M NH_3. The total tritium content and the 260 mμ extinction of each fraction (5 ml) were measured. Circles, $E_{260m\mu}$; triangles, radioactivity. (From Smith and Burton, 11).

TABLE I

FRACTIONATION OF PULSE-LABELED DNA WITH PHENOL

A culture of *E. coli* B/2 was infected with T5 phage at a multiplicity of 10 and labeled with ^{3}H-thymidine for 1 min at 35 min after infection. The collected bacteria were incubated with 0.7% sodium dodecyl sulfate and the lysate was rocked with water-saturated phenol and centrifuged. Acid-insoluble deoxyribose and tritium were measured in the resultant aqueous phase and interface precipitate.

Fraction	Total deoxyribose	Total radioactivity
	μmole	*CPM*
Aqueous phase	1.20	1,560
Interface	0.92	12,200

teria (11). In this case chloramphenicol, which inhibits condensation of precursor DNA to form phage particles, also inhibited the transfer of labeled DNA from the interfacial to the aqueous fraction.

The phenol fractionation provided a convenient method for measuring the sizes of the DNA pools after infection with T5 phage (Fig. 3) (11). The DNA content of the interface fraction increased to a constant level of about 50

phage DNA equivalents per bacterium by 30 min. DNA in the aqueous fraction began to increase at about 24 min. A similar study with T2-infected bacteria was complicated by the presence of bacterial DNA in the fractions, a difficulty that could be overcome by appropriate isotopic techniques.

Examination of the interface material in the electron microscope revealed

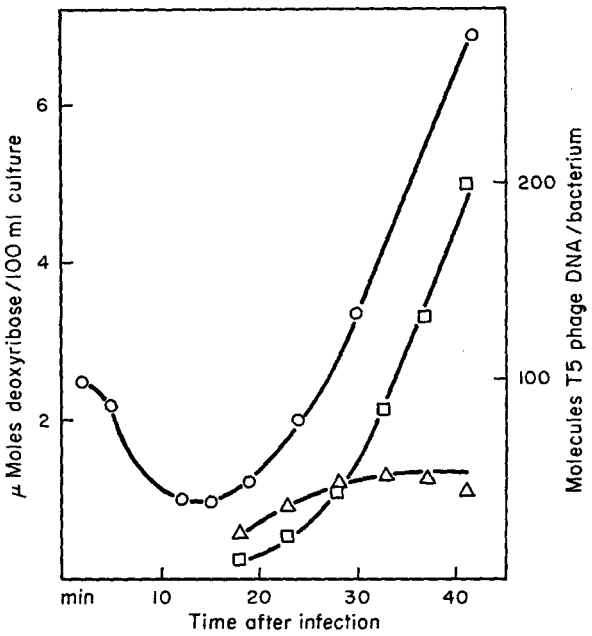

FIGURE 3. Changes in DNA content of the aqueous and interface fractions after infection with T5 phage. Samples were removed from a culture of T5-infected *E. coli* at the indicated times. After treatment with dodecyl sulfate the bacterial lysates were fractionated with phenol as described in Table I, and the DNA content of the aqueous phase and interface fractions was measured. Circles, total DNA in the culture; squares, aqueous phase fraction; triangles, interface fraction. (From Smith and Burton, 11.)

bacteria-like structures, from which DNA could be released with lysozyme or pronase but not with papain. DNA recovered in this way was firmly bound by methylated albumin columns (11). We therefore believe that replicating DNA, because of its large size or other special properties, is trapped within the rigid-layer framework of the cell, which is not degraded by detergent and phenol but is degraded by lysozyme (13). If so, DNA of phage particles must be able to pass through holes in this rigid-layer fabric. Although a significant proportion of the DNA can be released from the interfacial material with lysozyme alone, and the yield is not improved by the use of pronase, questions concerning possible attachment of replicating DNA to cellular structures remain open.

A similar difficulty in extracting DNA from T4-infected cells with detergent and phenol was encountered by Kozinski and Lin (14). They suggested that the DNA was associated with a bacterial protein. Trapping of replicating DNA within the rigid layer of partially lysed cells could also explain their results.

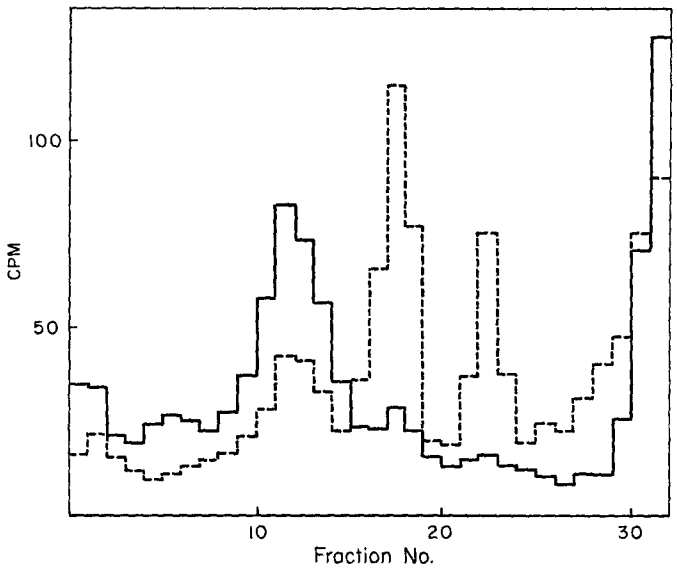

FIGURE 4. Sucrose density gradient centrifugation of DNA from λ-infected *E. coli*. A culture of *E. coli* was infected with ^{32}P-labeled λ-phage at a multiplicity of 6, and 22 min after infection the culture was labeled with ^{3}H-thymidine for 1 min. The bacteria were immediately collected and lysed with lysozyme and detergent, and the lysates were extracted with phenol. After dialysis, a sample of the DNA (0.15 μg) was layered on a 5 to 20% gradient of sucrose (containing 2 M NaCl and 0.05 M phosphate buffer, pH 6.7) and centrifuged for 3 hr at 30,000 RPM. Fractions were obtained by piercing the bottom of the tube and collecting drops directly into counting vials. Solid line, ^{3}H (pulse label); broken line, ^{32}P (parental).

Sedimentation Properties of Pulse-Labeled DNA Pulse-labeled DNA from both T5- and λ-infected bacteria sediments faster in a sucrose density gradient than DNA of purified phage particles. In an experiment with λ (Fig. 4) bacteria were infected with ^{32}P-labeled phage and then fed ^{3}H-thymidine for 1 min at 22 min after infection. The DNA sedimented in three components: (*a*) DNA sedimenting like that from phage particles; (*b*) the "supercoil" form of λ-DNA described by Young and Sinsheimer (15) and Bode and Kaiser (5), sedimenting 1.5 times faster than (*a*); and (*c*) a hitherto undescribed component, most of which sedimented twice as fast as (*a*) and some even faster. Most of the parental DNA was found in (*a*) and (*b*). Nearly all the pulse-labeled DNA was found in component (*c*).

In a similar experiment with T5 (Fig. 5) the bacteria were infected with ^{32}P-labeled phage and the culture was divided into two portions, which were fed ^{3}H-thymidine for 3 min at 25 and 30 min after infection, respectively. No fast-sedimenting parental DNA components were detected; most of the parental DNA sedimented at the rate of DNA from phage particles. On the other hand, much of the pulse-labeled DNA sedimented faster than DNA from phage particles and the sedimentation rate increased somewhat with

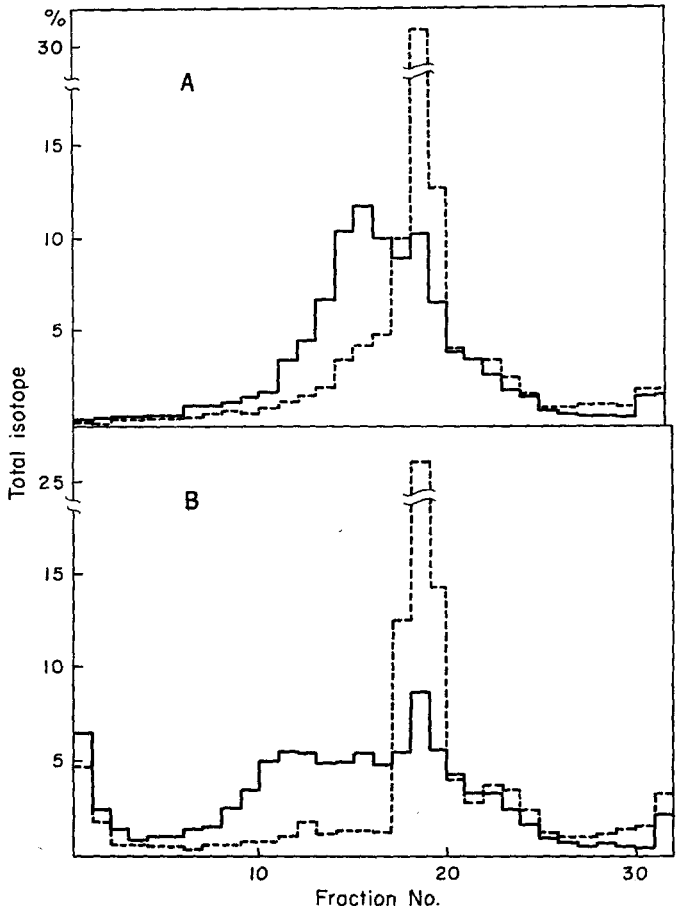

FIGURE 5. Sucrose density gradient centrifugation of DNA from T5-infected *E. coli*. A culture of *E. coli* was infected with ^{32}P-labeled T5 phage (multiplicity of infection, 7) and divided into two portions. These cultures were labeled with ^{3}H-thymidine for 3 min at, respectively, (*A*) 25 min and (*B*) 30 min after intection. Immediately after the pulse the bacteria were collected and the DNA isolated as described in Fig. 4. A sample (0.3 μg) of each DNA preparation was layered on a sucrose density gradient (5 to 20%, containing 2 M NaCl) and centrifuged for 2 hr at 35,000 RPM. Fractions were collected as described in Fig. 4 and counted. Solid line, ^{3}H (pulse label); broken line, ^{32}P (parental).

time after infection. Shorter pulses gave similar results except that a smaller fraction of the label was found at the position of DNA from phage particles.

The fast sedimentation of the pulse-labeled DNA was probably not due to nonspecific aggregation, for the following reasons. (*a*) Added phage DNA always sedimented normally. (*b*) The amount of DNA applied to the sucrose gradient (0.15 μg) should have been too small to encourage aggregation. (*c*) Heating the DNA almost to its melting temperature and cooling it quickly, which destroys known aggregates of λ-DNA (6), did not affect the sedimentation of pulse-labeled DNA. (*d*) Degradation by shear did not reveal a characteristic subunit (see below).

TABLE II

HYBRIDIZATION OF PULSE-LABELED DNA FROM λ-INFECTED *E. coli* WITH λ-PHAGE DNA AND *E. coli* DNA

A culture of λ-infected *E. coli* W3110 was labeled with ^{3}H-thymidine for 1 min at 25 min after infection. DNA was isolated from the cells as described in the text and a sample was mixed with ^{32}P-labeled DNA from purified λ-phage. The mixture was sonicated and the DNA annealed with (*a*) unlabeled *E. coli* DNA and (*b*) unlabeled λ-phage DNA contained in agar. All operations were performed as described by Cowie and Hershey (16), and the amount of labeled DNA specifically bound was measured.

Labeled DNA	Unlabeled DNA in agar	Labeled DNA bound	λ-DNA in preparation
		%	%
^{3}H (pulse-labeled)	*E. coli* W3110	19.7	89.1*
^{32}P (λ-phage)	*E. coli* W3110	11.0	
^{3}H (pulse-labeled)	$\lambda b^{+}c_{1}$	61.5	85.2
^{32}P (λ-phage)	$\lambda b^{+}c_{1}$	72.3	

* Assumes 80% efficiency of binding for *E. coli* DNA.

The following facts argue against the possibility of DNA-protein complexes. (*a*) After phenol extraction the DNA preparations contained less than 0.4% of the original cellular protein, and sedimentation experiments with pulse-labeled DNA from infected cells labeled before and after infection with ^{14}C-lysine did not reveal any association of DNA with the residual protein. (*b*) Treatment of pulse-labeled DNA with proteolytic enzymes (T5 with pronase, trypsin, and papain; λ with pronase) did not alter its sedimentation rate. (*c*) When pulse-labeled DNA from T5- and λ-infected cells was banded in CsCl, reisolated, and centrifuged in a sucrose density gradient, fast-sedimenting material was still present.

Since the λ-infected cultures contained uninfected cells, it was important to measure the amount of pulse-labeled *E. coli* DNA in the preparations. Hybridization (16) of ^{3}H–pulse-labeled DNA with unlabeled λ and *E. coli* DNA in

agar showed that at 26 min after infection 85% of the tritium pulse was contained in λ-DNA (Table II). This conclusion was verified by a pulse-chase experiment, which showed that the pulse-labeled DNA was indeed a precursor of DNA sedimenting at the characteristic rate of DNA from phage particles.

Precursor-product relationships are illustrated in Fig. 6. A culture of λ-infected bacteria was labeled with ^{3}H-thymidine for 1.5 min at 22 min following infection, after which uptake of radioactivity was stopped by addition

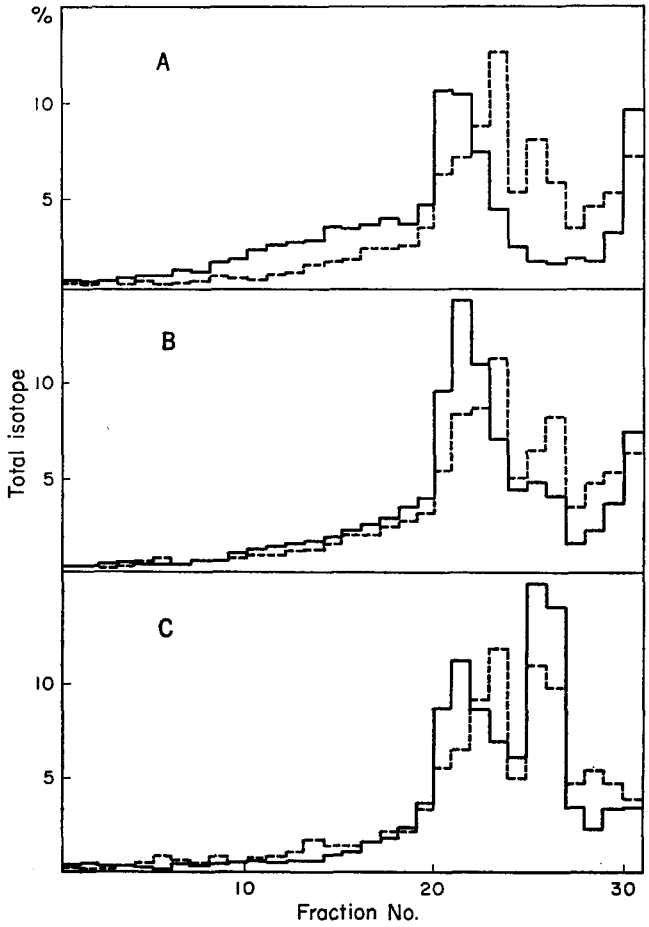

FIGURE 6. Precursor-product relationships of DNA from λ-infected *E. coli*. Experimental details were as described for Fig. 4, except that 1.5 min after addition of ^{3}H-thymidine a large excess of unlabeled thymidine was added and growth of the culture was continued. Samples were removed (*A*) immediately after addition of unlabeled thymidine and (*B*) 4 and (*C*) 8 min later. The DNA of each sample was isolated and centrifuged in sucrose density gradients for 1 hr at 35,000 RPM. Solid line, ^{3}H (pulse label); broken line, ^{32}P (parental).

of an excess of unlabeled thymidine. Samples of the culture were removed immediately after the pulse and 4 and 8 min later. Centrifugation of the extracts showed that during the 8 min period after the pulse a considerable proportion of the label moved from the fast-sedimenting components to DNA resembling that from phage particles. Furthermore, the supercoil form of λ-DNA did not appear to be an intermediate in the transfer. After infection we were never able to introduce appreciable amounts of label into this component. Parental label accumulated in it during the first 8 min, but thereafter the amount did not alter appreciably.

Further sedimentation studies were performed by treating the pulse-labeled

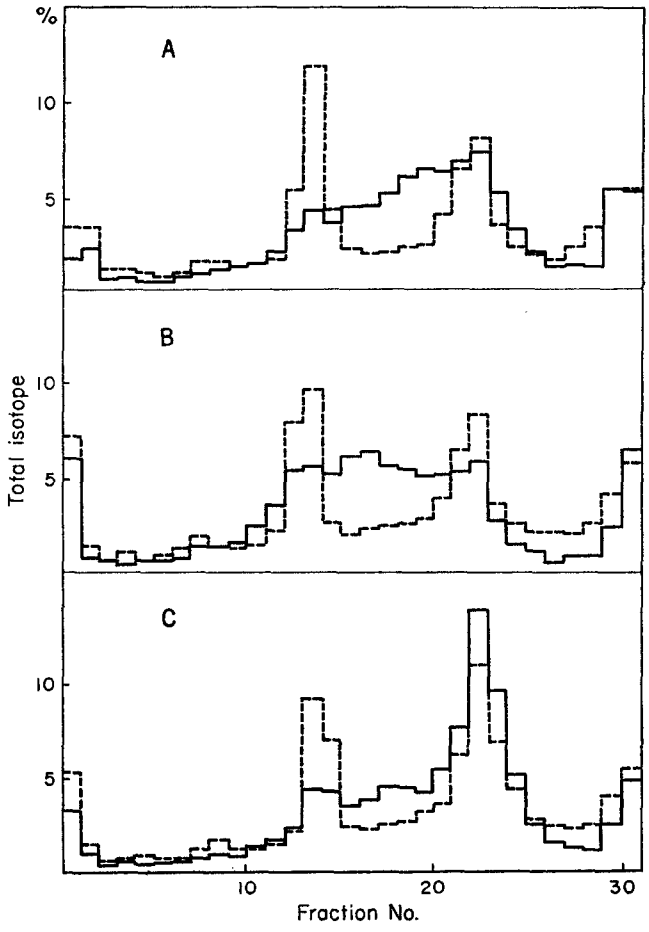

FIGURE 7. DNA from λ-infected *E. coli* centrifuged in alkaline sucrose. Samples of DNA from the pulse-chase experiment (Fig. 6) were treated with 0.1 M NaOH and centrifuged in sucrose gradients containing 0.1 M NaOH and 0.9 M NaCl for 80 min at 35,000 RPM. (*A*) Immediately after ^{3}H-thymidine pulse, (*B*) 4 min and (*C*) 8 min later. Solid line, ^{3}H (pulse label); broken line, ^{32}P (parental).

DNA of λ- and T5-infected bacteria with 0.1 M NaOH and centrifuging the denatured DNA in sucrose gradients containing 0.1 M NaOH and 0.9 M NaCl. In extracts from λ-infected cultures, labeled parental DNA appeared in two major components (Fig. 7), one at the position of intact single strands of DNA from phage particles, the other sedimenting approximately twice as fast and representing the collapsed supercoil described by others. A small fraction of parental DNA was found between these peaks. The pulse-labeled DNA, on the other hand, sedimented as a broad band between the two major

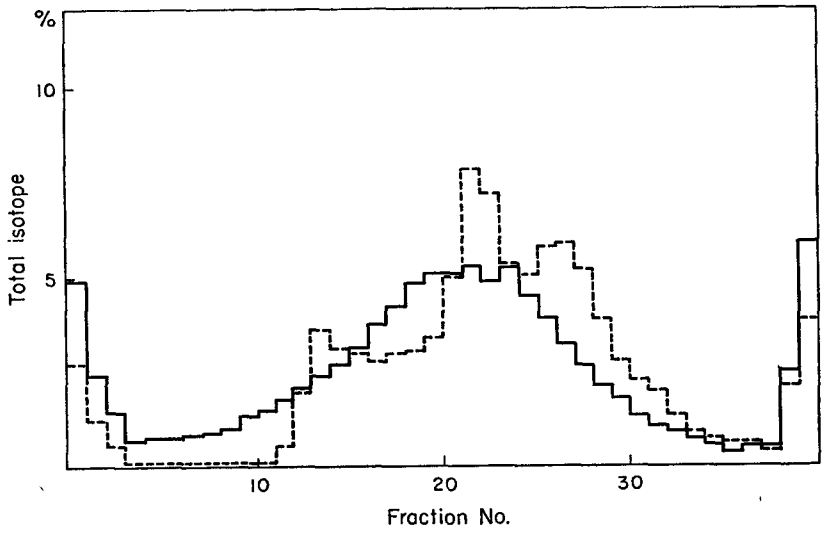

FIGURE 8. DNA from T5-infected *E. coli* centrifuged in alkaline sucrose. The culture was infected with unlabeled T5 phage and labeled with ^{3}H-thymidine for 1 min at 33 min after infection. A sample of the isolated pulse-labeled DNA was mixed with ^{32}P-labeled T5 phage DNA and treated with 0.1 M NaOH before centrifugation in a 5 to 20% sucrose gradient containing 0.1 M NaOH. Solid line, ^{3}H (pulse label); broken line, ^{32}P (phage).

parental components. Essentially the same results were obtained in sucrose solutions containing 0.3 and 0.5 M NaOH, except that the supercoil form sedimented faster than in solutions containing 0.1 M NaOH. Therefore a large proportion of the pulse-labeled polynucleotide chains were longer, more compact, or more difficult to separate than those found in phage particles.

After alkali treatment and neutralization, pulse-labeled DNA had the expected buoyant density for single-stranded DNA in cesium chloride. This result excludes certain types of cross-linking of DNA strands that prevent permanent denaturation.

Phage T5 DNA is unusual in that three discrete bands are obtained on sedimentation in alkali (Fig. 8), indicating that the molecule has single-strand

breaks at specific places (17). Pulse-labeled T5 DNA sediments in alkali at about the same rate as DNA from phage particles but does not resolve into individual components. These polynucleotide chain lengths may reflect nuclease action during isolation; or some of the breaks found in the finished molecule may already be present in its precursor.

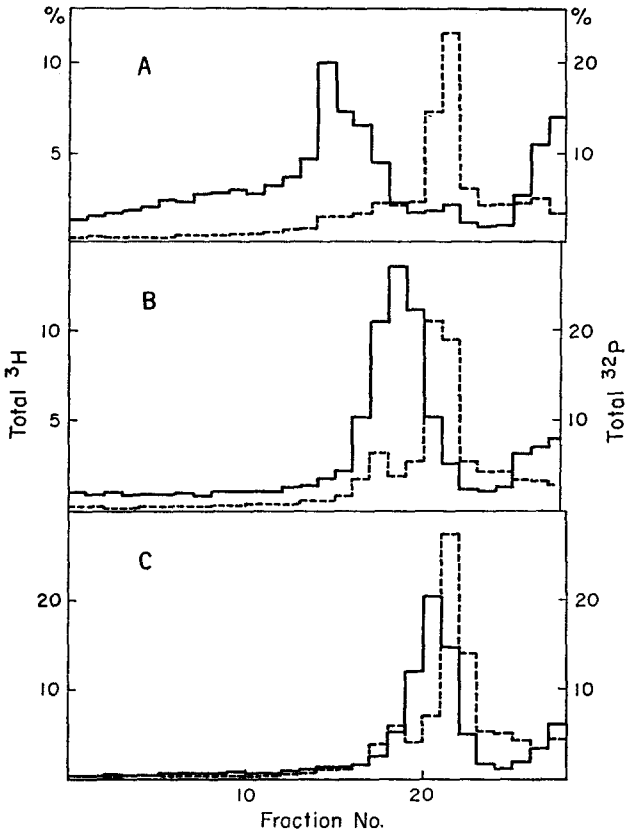

FIGURE 9. Breakage of λ–pulse-labeled DNA by shear. A sample of the DNA preparation described in Fig. 4 was mixed with ^{32}P-labeled λ-phage DNA. The mixture was passed several times through a narrow bore pipet, and portions were centrifuged in sucrose gradients as for Fig. 4. (*A*) Unsheared, (*B*) slow pipetting, (*C*) vigorous pipetting. Solid line, ^{3}H (pulse label); broken line, ^{32}P (phage DNA + parental).

Both in the λ and in the T5 system we found rapidly sedimenting phage precursor DNA. In general, rapid sedimentation implies high molecular weight or compact shape. A compact form of DNA is likely to be resistant to shear, whereas long molecules are characteristically fragile. The supercoil form of λ-DNA, for instance, is resistant to shear (18). Some effects of shear on λ–pulse-labeled DNA are illustrated in Fig. 9. Passing a mixture of pulse-labeled

DNA and differently labeled marker DNA from phage particles through a small bore pipet reduced the sedimentation rate of pulse-labeled DNA from 2.0 to 1.4 times that of the marker. Somewhat higher shear broke the pulse-labeled DNA into pieces sedimenting at 1.24 times the rate of the marker. Neither treatment affected marker DNA itself. Similar results were obtained by controlled stirring; they are shown in Table III. At very high stirring speeds (3000 RPM) both the pulse-labeled and the marker DNA broke into pieces about half the original size of the marker. Thus pulse-labeled λ-DNA is much more sensitive to shear than DNA from phage particles, and can be

TABLE III

SEDIMENTATION RATE OF PULSE-LABELED DNA FROM λ-INFECTED *E. coli* AFTER SHEARING

DNA was obtained from λ-infected *E. coli* as described in Fig. 4. A sample of this ^{3}H-labeled DNA was mixed with ^{32}P-labeled λ-phage DNA, and aliquots were stirred at the designated speeds for 30 min at 5°. The sedimentation properties of the stirred DNA were examined by sucrose density gradient centrifugation. The table shows the mean sedimentation rate of the breakage products as a function of that of unbroken λ-phage DNA.

Stirring speed	Sedimentation rate
RPM	(× *S of* λ-*DNA*)
Unstirred	2.0*
400	2.0
800	1.6
1200	1.4
1600	1.26
2000	1.05
3000	0.77

* Also contained faster sedimenting material.

reduced to any of a number of relatively homogeneous products depending on shear rate, but not to any characteristic subunit. This is the behavior expected for very long DNA molecules. It is not readily accounted for on the basis of aggregates of smaller units or compact forms of a single class.

Although shearing experiments with T5 pulse-labeled DNA were less conclusive, the pulse-labeled DNA was at least as fragile as DNA from phage particles.

The results described here with λ–pulse-labeled DNA are very similar to those obtained by Frankel (19) with a small fraction of DNA recovered from cultures of T2-infected bacteria treated with chloramphenicol. Frankel concluded that his material consisted of long linear molecules. More recently, by methods similar to ours, he has isolated from T4-infected *E. coli*, DNA that sediments considerably faster than DNA from phage particles (20).

Since isolation of DNA from bacteria inevitably involves shear, the phage

precursor DNA we have described may be a degradation product of still larger and more fragile structures. According to the sedimentation coefficient–molecular weight relationship of Burgi and Hershey (21), the bulk of the λ–pulse-labeled DNA (Fig. 4) has a molecular weight of 220 to 250 million, with faster-sedimenting components having a molecular weight of 500 million or more. Bacterial DNA in our extracts also had a molecular weight of 220 to 250 million, which is presumably the size determined by the extraction procedure. It therefore seems probable that the pulse-labeled material sedimenting at the rate appropriate to molecules of molecular weight 250 million was itself a product of shear, and that the material sedimenting still faster represented less fragile complexes of some sort. Preliminary experiments indicate that it is possible to obtain even faster-sedimenting pulse-labeled DNA by using more gentle isolation procedures.

The results of our experiments can be summarized as follows. Phage precursor DNA's isolated from bacteria infected with T5 or λ-phage were clearly different from DNA of the phage particles, and they were also different from each other. The λ-precursor DNA sedimented very fast in neutral sucrose solution and at the same rate as bacterial DNA present in the extract. After denaturation of the λ-precursor DNA had occurred (as shown by changes in density), a significant proportion of it sedimented faster in alkaline sucrose solution than phage DNA polynucleotide chains. After exposure to progressively increasing rates of shear the precursor DNA fragmented into pieces sedimenting at progressively slower rates. Except after very high shear, when the marker DNA also was broken, the breakage products of the λ-precursor DNA sedimented faster than unbroken phage DNA. The T5 precursor DNA was somewhat different. Although at neutral pH much of it sedimented faster than phage DNA, it was more heterogeneous, and in alkali it sedimented more slowly than some of the phage polynucleotide chains. These differences may be a result of more nuclease action in lysates of T5-infected than of λ-infected bacteria, or they may reflect genuine differences between the DNA's.

Many of the observed properties of precursor DNA could be due either to a a specific association of DNA with other cellular constituents or to changes in structure of the DNA. On the basis of experiments designed to examine the first possibility it seems unlikely that the DNA is physically associated with protein, RNA, or in the case of λ with bacterial DNA. If, as we think, the sedimentation properties of λ-precursor DNA result from an unusual structure, the simplest hypothesis consistent with our experimental results (especially the effects of shear) is that precursor DNA molecules are linear and considerably longer than the DNA of phage particles. Other, more complicated models can, of course, be devised.

Since the structure of the phage precursor DNA is pertinent to elucidation

of replicative and genetic processes, we hope that further work will allow more definitive conclusions.

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Discussion

Dr. Robert L. Novak: I'd like to know something about the nature of these bag-like structures at the interphase that contained the replicating DNA. Are these cells?

Dr. Smith: Yes. Some years ago Bolle and Kellenberger showed that treatment of *E. coli* with sodium dodecyl sulfate does not lead to a complete lysis or disruption of the cell, although bacterial suspensions become transparent. Under the electron microscope, the treated cells still had the same shape as intact bacteria. A similar observation has been reported by Wiedel, Frank, and Martin who showed that the rigid layer of the bacterial cell wall remains intact after treatment with detergent and phenol but can be degraded with lysozyme. Does this answer your question?

Dr. Novak: Yes. The question was the statement that the nonreplicating or completed DNA is able to get out of these partially lysed bacteria.

Dr. Smith: Well, we know the completed DNA gets out, but we don't know how it happens. We can only assume that there are holes which allow passage of this DNA. The replicating DNA which perhaps may also be involved in genetic recombination and messenger RNA synthesis and therefore have an unusual configuration is retained. This is the observation, but we don't really know what is the reason for it.

Dr. Harold Fisher: You mention that the fast sedimenting form is free of protein. Is it also free of RNA?

Dr. Smith: All I can say is the treatment of this material with ribonuclease doesn't alter the sedimentation pattern.

Question from the Floor: Have you looked for an association of RNA polymerase with the replicating form?

Dr. Smith: The answer is no. In experiments in which the bacteria were prelabeled with ^{14}C-lysine, we have not been able to show any association of the radioactive amino acid with the pulse-labeled DNA.