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Intracellular Forms of λ Deoxyribonucleic Acid in *Escherichia coli* Infected with Clear or Virulent Mutants of Bacteriophage λ

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ABSTRACT

WEISSBACH, ARTHUR (National Institutes of Health, Bethesda, Md.), ALLAN LIPTON, AND ARNOLD LISIO. Intracellular forms of λ deoxyribonucleic acid in *Escherichia coli* infected with clear or virulent mutants of bacteriophage λ. J. Bacteriol. **91**:1489–1493. 1966.—Infection of either the sensitive or lysogenic strain of *Escherichia coli* K-112S by λ^+ leads to the formation of a new phage deoxyribonucleic acid (DNA) species having the properties of a twisted circular DNA duplex. This new phage DNA species is also seen in cells infected with clear or virulent mutants of λ which cannot lysogenize, or do so at a low frequency. The sedimentation rate of circular λ DNA duplex at various *p*H values and its lability were examined.

The infection of either lysogenic or sensitive (nonlysogenic) cells of *Escherichia coli* K-12 with phage λ leads to a rapid intracellular formation of a new species of the phage deoxyribonucleic acid (DNA) (1*a*, 10). Bode and Kaiser (1*a*) have shown the presence of three differently sedimenting forms of the phage DNA isolated from the host cells. Centrifugation in sucrose gradients, at *p*H 7.4, revealed species I, sedimenting 1.9 times faster, and species II, sedimenting 1.14 times faster than the normal linear duplex DNA molecule, species III. At *p*H 12, species I sediments 3.6 times faster than denatured λ DNA, whereas species II sediments at the same rate as the latter.

Bode and Kaiser (1*a*) suggested that DNA species I represents a condensed circular form of λ DNA similar to the twisted circular form of polyoma viral DNA (9), that species II is a closed circular molecule, and that species III is the normal linear duplex DNA molecule which is the same as that obtained from mature λ phage. Species II seems to arise by spontaneous decay of species I.

The occurrence of circular forms of λ DNA in cells infected with this phage may be of some relevance to the mechanism by which λ establishes a lysogenic state. Campbell (2) has proposed a circular λ chromosome as an intermediate in the attachment of the λ DNA to the bacterial chromosome and formation of prophage. In view of the Campbell model and the above results indicating the presence of circular forms of λ DNA in infected cells, it became of interest to determine whether mutants of λ that are unable to lysogenize are also unable to produce the rapidly sedimenting circular species of DNA after infection. For this purpose, we have examined clear mutants of λ having mutations in the C_I, C_{II}, or C_{III} cistrons and λ_{vir}, a multiple mutant of λ having one of its mutations in the C_I cistron. These clear and virulent mutants of λ show little or no ability to lysogenize *E. coli* K-12 (1, 3).

This paper will show that infection of sensitive or lysogenic *E. coli* with clear or virulent mutants of λ causes the formation of the same rapidly sedimenting DNA form (species I) which is seen after infection with wild-type λ (λ^+).

MATERIALS AND METHODS

Bacterial and bacteriophage strains. E. coli K-112(S) (nonlysogenic) and K-112(λ_{22}) (a wild-type lysogen) have been previously described (6, 7) as has λ_{vir}. The following clear mutants were obtained from Vernon Bode: λ C_{72} (C_I mutant), λ C_{68} (C_{II} mutant), and λ C_{67} (C_{III} mutant). Preparations of these clear mutants were made from single plaque isolations. *E. coli* CR-34(S) (nonlysogenic) and CR-34 (λ) were obtained from M. Meselson.

Preparation of H^3- or C^{14}-λ. λ^+-E. coli CR-34(λ)

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was grown in synthetic medium containing (per liter): 10 ml of 10% Casamino Acids, 5 ml of 0.5 M $MgCl_2$, 2 ml of 0.5 M $CaCl_2$, 1.0 ml of thiamine hydrochloride (500 μg/ml), 3.0 ml of thymine (20 μmoles/ml), 4 ml of 0.5% leucine, 2.0 ml of 1% L-threonine, and 1 to 1.5 mc of H^3-thymine (New England Nuclear Corp., Boston, Mass.). The cells were grown with rapid aeration at 37 C and induced with mitomycin C when the cell population was 2×10^8 cells per milliliter (6). After lysis had occurred, the λ phage was isolated as described by Kaiser and Hogness (4) and banded twice in CsCl. For the preparation of C^{14}-λ, C^{14}-thymine was substituted for H^3-thymine.

Preparation of C^{14}-λ DNA. Purified λ phage, labeled with C^{14}-thymine, was shaken with phenol, and the DNA was isolated as previously described (8).

Preparation of H^3 C mutants and λ_{vir}. E. coli CR-34-(S) was grown in synthetic medium containing H^3-thymine as described above. When the cell density reached 2×10^8 per milliliter, the culture was infected with the appropriate λ mutant at a multiplicity of infection of one phage per 10 cells. After lysis had occurred, the phage were isolated by two cycles of low- and high-speed centrifugation (7) and banded two times in CsCl.

Infection of cells with H^3-λ phages. E. coli K-112S [or K-112(λ_{22})] was grown in 500 ml of synthetic medium at 37 C (5). At a cell density of 2×10^8 per milliliter, the culture was infected with the appropriate H^3-λ strain at a multiplicity of five phage per cell. (Under these conditions over 90% of the cells lysed, with a burst size of 100 to 200 phage per cell.) At 15 min after infection the culture was rapidly cooled to 0 C, and the cells were collected by centrifugation. The cells (0.5 g) were washed once with 0.01 M tris(hydroxymethyl)aminomethane (Tris), *p*H 8.0, and either stored at −120 F or immediately used for the DNA isolation described below.

DNA isolation from infected cells. A modification of the procedure described by Bode and Kaiser (1*a*) was used. A 0.5-g amount of washed cells was suspended at 0 C in 4 ml of 1.0 M sucrose in 0.01 M Tris, *p*H 8.0. A 2-ml amount of 0.1% lysozyme was added, followed in 5 min by 2 ml of 0.2 M ethylenediaminetetraacetic acid (EDTA), *p*H 8.0. After an additional 5 min, 12 ml of 0.75% sodium dodecyl sulfate in 0.01 M Tris plus 0.001 M EDTA (*p*H 8.0) was added, and the suspension was kept at 0 C for 30 min. This suspension was shaken once with an equal volume of phenol (water-saturated with 0.01 M Tris plus 0.001 EDTA, *p*H 8.0) for 3 min, and the aqueous phase was removed by centrifugation. The phenol layer was washed once with 10 ml of 0.01 M Tris (*p*H 7.4), and the aqueous phases were combined and dialyzed against 10^{-3} M Na_3PO_4 for 18 hr at 2 C. The dialyzed preparations were concentrated in vacuo by lyophilization to a final volume of 3 to 5 ml. The preparations so obtained were usually turbid and, in some cases, were shaken again with an equal volume of water-saturated phenol two to four times, redialyzed against 0.01 M Na_3PO_4 for 6 hr, and concentrated by lyophilization, if necessary, to 3 to 5 ml. These preparations contained 20,000 to 40,000 counts per min per ml and were centrifuged directly in the sucrose gradients as described below.

Sucrose gradient centrifugation. A 29-ml amount of a linear 10 to 30% sucrose gradient was placed over 1 ml of 50% sucrose, and 1 ml of the preparation to be analyzed was layered on top. The sucrose solutions were prepared either in 0.01 M Tris (*p*H 7.4) or in 0.02 M Na_3PO_4. In the latter case, the *p*H meter readings were 11.1 (10% sucrose) and 10.6 (30% sucrose). In some experiments, the 10 and 30% sucrose solutions in 0.02 M Na_3PO_4 were brought to *p*H 12.2 by the addition of NaOH.

Centrifugation was carried out in a Spinco SW-25 head for 16 hr at 40,692 × *g* at 0 C. At the end of the centrifugation, tubes were punctured at the bottom, and 1-ml fractions were collected. For radioactive measurements, 0.20 ml of salmon sperm DNA (2.5 mg/ml) and 2 ml of water were added to each tube, and the DNA was precipitated by the addition, with stirring, of 0.5 ml of 6% perchloric acid. The precipitate was collected by centrifugation and dissolved in 0.50 ml of hyamine hydroxide (Packard Instrument Co., La Grange, Ill.) at 50 C for 10 min. The hyamine solutions were placed in 10 ml of toluene scintillator (8), and were counted in a liquid scintillation spectrometer.

RESULTS

Control experiments. Figure 1 shows the results of control experiments designed to locate the

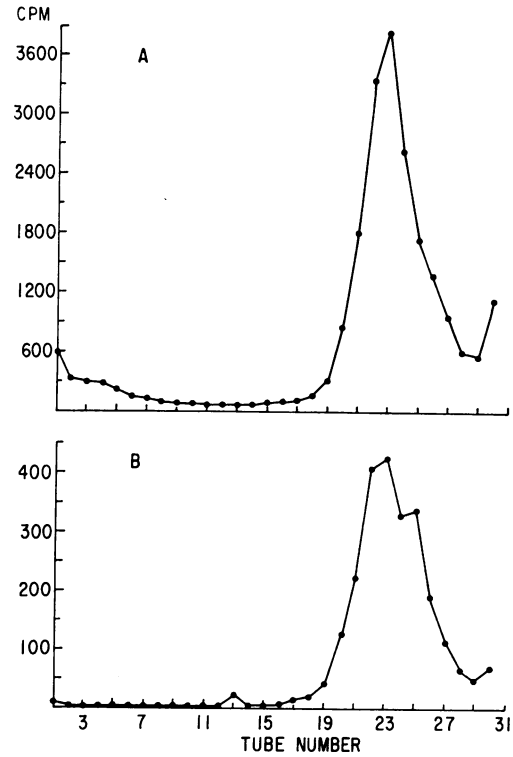

FIG. 1. *Sedimentation of λ-DNA; 10 to 30% sucrose gradients in 0.01 M Tris (pH 7.4) were used. (A) DNA from C^{14}-λ^+ phage. (B) DNA preparation from cells infected at 0 C with H^3-λ^+.*

position of λ DNA in 10 to 30% sucrose gradients after 16 hr of centrifugation at 40,692 × *g*. In Fig. 1A, a preparation of C^{14}-λ DNA obtained by phenol treatment of C^{14}-λ phage shows a single peak migrating about one-fourth of the way down the tube. In another control experiment, *E. coli* K-112S cells were mixed with H^3-λ (C_{II} preparation) at 0 C under conditions similar to those used for active phage infections at 37 C (*see* Materials and Methods). Under these conditions, the λ phage absorbs to the cells but does not inject its DNA into the cell (1*a*). The DNA, prepared from these cells infected at 0 C, also shows one peak in the sucrose gradient (Fig. 1B), sedimenting at the same rate as normal λ DNA obtained directly from λ phage. The results are the same whether the centrifugations are carried out in Tris at *p*H 7.4 or in Na_3PO_4, *p*H 10.6 to 11.1.

Infection of cells with wild-type λ. The infection of the wild-type lysogen *E. coli* K-112 (λ_{22}) at 37 C with λ^+ leads to the intracellular formation of another DNA species sedimenting about two times as fast as normal phage DNA (Fig. 2A). The results are similar to those described by Bode and Kaiser. The experiment in Fig. 2A was carried out in sucrose in 0.01 M Tris (*p*H 7.4). A similar experiment was performed by use of a sucrose gradient in 0.02 M Na_3PO_4 having a *p*H range from 10.6 to 11.1. Figure 2B shows that the results are essentially the same as those obtained at *p*H 7.4. Since we have found that similar DNA sedimentation patterns are obtained with sucrose dissolved either in 0.01 M Tris (*p*H 7.4) or 0.02 M Na_3PO_4 (*p*H range, 10.6 to 11.1), we have used both of these conditions in our experiments.

The infection of the nonlysogenic sensitive strain *E. coli* K-112S with λ^+ also led to the formation of a similar rapidly sedimenting form of λ DNA (Fig. 3). The relative amount of the rapidly sedimenting form of the λ DNA was less than that seen in lysogenic cells. This may be due to the variable degradation of the rapidly sedimenting DNA species in the isolation procedure.

Bode and Kaiser (1*a*) have recently reported that the rapidly sedimenting form of the DNA (species I) is converted in 0.3 N NaOH to a form sedimenting 3.6 times as fast as normal denatured λ DNA. We found the same results (Fig. 3) when the same DNA preparation obtained from a λ^+ infection of K-112S was centrifuged in sucrose dissolved in 0.02 M Na_3PO_4 at either *p*H 10.6 to 11.1 or at *p*H 12.2. At *p*H 10.6 to 11.1, the usual rapidly sedimenting peak moving two times as fast as normal DNA was noted; at *p*H 12.2, a DNA form sedimenting 3.5 times as fast as denatured DNA was found with no sign of

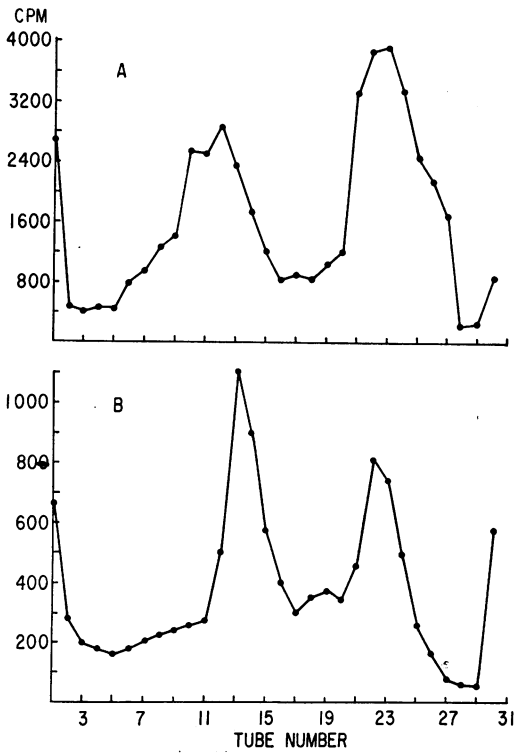

FIG. 2. *Sedimentation of DNA from lysogenic cells infected with H^3-λ^+. Sucrose gradient centrifugation was performed in 0.01 M Tris at pH 7.4 (A), or in 0.02 M Na_3PO_4 at pH 10.6 to 11.1 (B).*

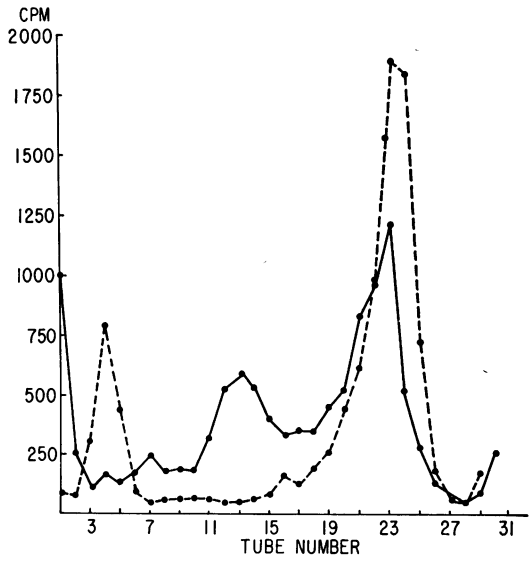

FIG. 3. *Sedimentation of DNA from nonlysogenic cells infected with H^3-λ^+. Sucrose gradient centrifugation was carried out at pH 10.6 to 11.1 (solid line) or at pH 12.2 (dashed line) in 0.02 M Na_3PO_4.*

the fast-moving form seen at *p*H 10.6 to 11.1 (or at *p*H 7.4).

Infection of cells with λ C mutants and λ_{vir}. Figure 4 shows the results obtained when *E. coli* K-112(S) is infected with various C mutants of λ. The experimental conditions were the same as those employed when cells were infected with λ^+. The C_I, C_{II}, and C_{III} mutants of λ all formed the rapidly sedimenting form of the phage DNA after infection of the host cell. Again, the different relative amounts of the rapidly sedimenting DNA detected in these experiments may reflect the isolation procedure. Thus, the C_I and C_{III} DNA preparations of Fig. 4 had received just one treatment with phenol, whereas the C_{II} preparation was shaken five times with phenol. This difference in the number of phenol treatments with the various DNA preparations received may also explain the variation in the sharpness and mobility of the DNA peaks seen in the sucrose gradients. As shown in Fig. 5, λ_{vir}, a multiple mutant containing a mutation in the C_I region, also forms the rapidly sedimenting DNA after infection of *E. coli* K-112 (λ_{22}).

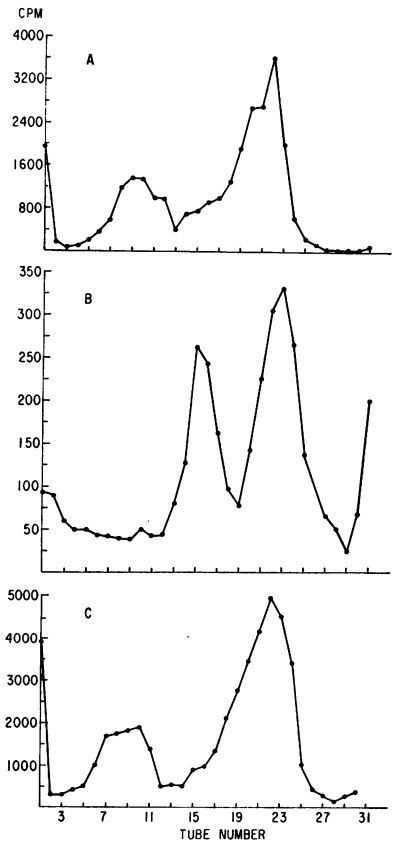

FIG. 4. *Sedimentation of DNA from nonlysogenic cells infected with H^3 clear mutants of λ. (A) C_I, (B) C_{II}, (C) C_{III}. Sucrose gradients at pH 10.6 to 11.1 in 0.02 M Na_3PO_4 were used.*

Lability of rapidly sedimenting DNA. In these particular experiments, approximately one-third of the infecting λ DNA was found as the rapidly sedimenting form after isolation from infected cells. This may be a minimal amount, since the rapidly sedimenting DNA form is labile and quickly converts back to a form sedimenting at the same rate as normal linear λ DNA. In our experiments, incubation of DNA preparations containing the rapidly sedimenting form in 0.01 M Tris (*p*H 7.4) at 37 C led to the disappearance of approximately 50% of this species in 20 min and almost complete disappearance in 60 min. Storage of the DNA preparations at −15 C for 1 month also caused the loss of about two-thirds of the rapidly sedimenting DNA form. Bode and Kaiser (1*a*) found little if any of their species I after storage at 5 C for 4 months. The degradation and loss of the rapidly sedimenting form may be due to an inherent instability of this species or to trace amounts of nucleases still present in these DNA preparations.

DISCUSSION

The results reported in this paper agree with the findings reported by Bode and Kaiser (1*a*) that a rapidly sedimenting form of λ DNA is formed in lysogenic cells infected with λ^+. We have also found this to be true in nonlysogenic cells infected with λ. Though Bode and Kaiser were able to detect three DNA species by their technique, our method can only distinguish the fastest moving form of the phage DNA (species I) from the normal λ DNA (species III). DNA

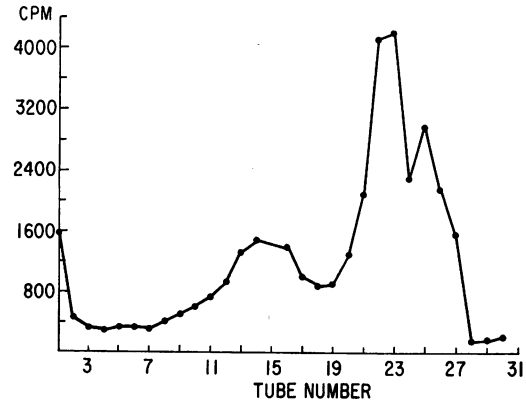

FIG. 5. *Sedimentation of DNA from lysogenic cells infected with H^3-λ_{vir}. A sucrose gradient in 0.01 M Tris (pH 7.4) was used.*

species II, which has a sedimentation rate only slightly greater than species III, cannot be recognized in our experiments. There is an apparent discrepancy between the results of Young and Sinsheimer (10) and those of Bode and Kaiser as to the relative sedimentation rate of the fast-moving form of λ DNA at *p*H 12. The former workers found the sedimentation rate of the fast-moving DNA form to be about two times that of λ DNA in gradients run in 0.02 M Na_3PO_4 (*p*H 12.2), whereas the latter group found it to be 3.6 times that of normal denatured DNA. In our experiments with sucrose gradients in 0.02 M Na_3PO_4 titrated with NaOH and having *p*H meter readings of 12.2, we found the mobility of the rapidly sedimenting DNA form to be the same as that reported by Bode and Kaiser. The use of sucrose gradients in 0.02 M Na_3PO_4, which have *p*H meter readings of 10.6 to 11.1 and have not been brought to *p*H 12 with NaOH, results in a rapidly sedimenting form moving two times as fast as normal λ DNA, which is the same as the mobility at *p*H 7.4. If the sucrose gradients used by Young and Sinsheimer were in fact at a *p*H somewhat below 12.2, their results would agree with those of Bode and Kaiser.

The clear and virulent mutants of λ are unable to lysogenize *E. coli* K-12, even at a low frequency. A reasonable hypothesis, based on the Campbell (2) model of lysogenization, would be that such mutants would be unable to form the rapidly moving, circular form of phage DNA. However, the present experiments show that the C mutants of λ do form the fast-moving, condensed circular form of DNA when they infect the nonlysogenic *E. coli* K-112(S). Infection of the lysogenic K-112 (λ) with a C_{III} mutant has given the same results, and one might anticipate this to be true with the other clear mutants of λ. Similarly, λ_{vir} infection of either sensitive or lysogenic cells leads to the formation of the rapidly sedimenting DNA form.

The possible function of the rapidly sedimenting twisted circles of phage DNA which arise after infection of host cells is obscure. A circular DNA duplex fits into the Campbell (2) model of lysogenization. Our experiments with the λ mutants which are defective in their ability to lysogenize do not negate or support this model, but merely show that these mutants are capable of forming the circular DNA duplex when they infect a host cell.

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