JOURNAL OF VIROLOGY, Aug. 1977, p. 377-383

Vol. 23, No. 2
Printed in U.S.A.

Fragmentation of *Bacillus* Bacteriophage ϕ105 DNA by Restriction Endonuclease *Eco*RI: Evidence for Complementary Single-Stranded DNA in the Cohesive Ends of the Molecule

BARBARA M. SCHER, DONALD H. DEAN, AND ANTHONY J. GARRO*

Department of Microbiology, Mount Sinai School of Medicine of The City University of New York, New York, New York 10029; and Department of Microbiology, Ohio State University, Columbus, Ohio 43210*

Received for publication 8 March 1977

The structure of DNA from the temperate *Bacillus subtilis* phage ϕ105 was examined by using the restriction endonuclease *Eco*RI and by sedimentation analysis. The DNA contains six *Eco*RI cleavage sites. Although eight DNA fragments were identified in the *Eco*RI digests, the largest of these was shown to consist of the two fragments that carry the cohesive ends of the phage DNA. In neutral gradients, the majority of whole ϕ105 DNA sedimented as nicked circles and the remainder as oligomers. No unit-length linear structures were detected. The associated cohesive ends could be sealed by DNA ligase from *Escherichia coli* and could be cleaved by S1 nuclease. On the basis of these results and previously reported studies, it appears that, as isolated from phage particles, ϕ105 DNA is a circular molecule that is formed from the linear structure by the association of complementary single-stranded DNA.

Studies of temperate bacteriophages have provided useful insights into the mechanisms by which viral genomes and other types of extrachromosomal DNA interact with the chromosomes of their hosts' cells. A good example of this is the model developed by Campbell (6) to explain the process of site-specific integration and excision typical of the lambdoid phages. This model depicted the integrative form of the λ genome to be a circular molecule that opened at a single point, the *att* site, during recombination with the *Escherichia coli* chromosome. Since the DNA was lost from either the phage or the cell, the model provided an explanation for the reversibility of this recombination and for the ability to isolate viable cells cured of the prophage. Also, by placing the *att* site toward the middle of the linear form of the phage genome, the model explained the permutation in gene order that had been observed in comparisons of the vegetative and prophage genetic maps.

To date, only two infectious phages have been described whose integration-excision cycles appear to differ significantly from the above model. One of these is the mutator phage Mu, which integrates at a large number of sites, if not randomly, into the *E. coli* chromosome (see reference 9 for review); the other, the subject of this report, is the *Bacillus subtilis* phage ϕ105. Although only a single integration site has been reported for ϕ105, it does share a number of similarities with Mu. First, the *att* sites of both phages appear to involve the ends of the viral genomes (8, 9, 26). Whether these ends act as two separate *att* sites, in which case integration would cause the loss of some bacterial DNA, or whether the ends join to form a single *att* site is still uncertain. Second, after induction of prophage replication, both ϕ105 and Mu replicate as complexes of phage and bacterial DNA (21, 23).

Clues for unraveling the integration-excision mechanisms of these phages have been sought in structural studies of the phage DNAs. These studies have revealed several unusual features of Mu DNA such as the heterogeneous end sequences which are, in fact, bacterial DNA and the presence of an invertible 3-kilobase-long sequence within the molecule (9). ϕ105 DNA has not been studied as extensively as Mu DNA. The conclusions drawn from initial studies were that ϕ105 DNA, as extracted from the phage particle, was a linear duplex that had the capacity to cyclize but did not appear to have λ-like single-stranded ends (2, 3). This latter point was based both on the absence of a detectable increase in circular or dimeric forms after exposure of ϕ105 DNA to annealing conditions and on the resistance to inactivation of ϕ105 DNA biological activity by exonuclease I. More recent studies have been interpreted as

indicating that ϕ105 DNA does have λ-like cohesive ends but, either because of shorter length or higher adenine-thymine composition, the end-join formed is not as stable as that of λ DNA (7). These conclusions were drawn from the high frequency of circular molecules seen by electron microscopy in preparations of denatured-renatured ϕ105 DNA and the relative sensitivity of these circles to disruption by formamide. Alternatively, the ends of ϕ105 DNA may associate through a protein link, as has been observed for the *Bacillus* phage ϕ29 and some adenoviruses (16, 22). In fact, transfection with ϕ105 DNA was reported to be protease sensitive but to a lesser degree than transfection by ϕ29 DNA (H. Hirokawa, Microbiol. Gen. Bull. no. 37, p. 8, 1974). Because of the importance of the ends of the ϕ105 DNA molecule to prophage integration, the goals of the present study were to identify, in restriction enzyme digests, the fragments derived from these ends and to gain additional information regarding their structure and ability to associated with each other.

MATERIALS AND METHODS

Phage and bacterial strains. Wild-type and *sus* mutant strains of ϕ105 were from L. Rutberg. The host that was lysogenized by the wild-type phage from which ϕ105 DNA was isolated was *B. subtilis* 168 strain GB75. This strain is noninducible for the defective phage PBSX (33). The ϕ105 *sus* mutants were assayed on strain MB228, which carries the su^{+3} suppressor, and 44AO was used as the nonpermissive host. The strain used as the host for marker rescue experiments was *B. subtilis* GB7044. *E. coli* CHS 45, which is lysogenized by λ*c*I857*sus*S7, was from the Cold Spring Harbor Laboratory collection. T7 and its host, *E. coli* O11′, were from F. W. Studier; the lysis-delay mutant ϕ29*sus*14(1241) and *B. subtilis* SpoA12 were from B. Reilly.

Chemicals and enzymes. Agarose (electrophoretic grade) and mitomycin C were purchased from Sigma Chemical Co., ethidium bromide and Pronase from Calbiochem, and proteinase K from E. M. Laboratories. The restriction endonuclease *Eco*RI was obtained from Miles Laboratories Inc., and *E. coli* ligase was from New England Biolabs. Nuclease S1 of *Aspergillus oryzae* was the generous gift of J. Wetmur. All other enzymes were from Worthington Biochemicals Corp.

Preparation of phage DNA. Bacteriophage ϕ105 was prepared by induction of *B. subtilis* GB75(ϕ105). Mitomycin C was added at a concentration of 0.4 μg/ml to cultures grown to a density of 5×10^8 CFU/ml in VY broth (13). After 120 min of incubation at 37°C, a drop of $CHCl_3$ was added and the phage was concentrated by polyethylene glycol precipitation (37). The phage was purified by banding in a CsCl step gradient prepared in phage diluent containing 0.01 M Tris (pH 7.4), 5×10^{-3} M $MgCl_2$, 2×10^{-5} M $MnCl_2$, and 0.01 mg of gelatin per ml. The gradient layers consisted of 3 ml of 1.65-g/cm³ CsCl, 4 ml of 1.43-g/cm³ CsCl, and 5 ml of the concentrated phage resuspended in phage diluent. The layered gradient was centrifuged for 16 h at 33,000 rpm and 10°C in a Spinco SW41 rotor. The purified phage was dialyzed overnight against 2,000 volumes of phage diluent. Phage ϕ29 was prepared by infection of *B. subtilis* SpoA12. The media and purification procedures were as described by Ito et al. (16). Phage λ was purified from lysates of a thermally induced *c*I857*sus*S7 lysogen by the method of Hedgpeth et al. (14).

DNA was extracted from the purified phages by gentle mixing with an equal volume of phenol that had been equilibrated with buffer (0.15 M NaCl, 0.001 M EDTA, 0.006 M Na_2HPO_4, 0.002 M NaH_2PO_4, 0.1 M mercaptoethanol, pH 7.4). Phenol was removed from the DNA preparations by dialysis against 0.015 M NaCl-0.0015 M sodium citrate, pH 7.0, at 4°C.

Preparation of radioactively labeled DNA. Tritiated phage ϕ105 was prepared by mitomycin C induction, as described above, using VY medium supplemented with 250 μg of deoxyadenosine and 5 μCi of [^{3}H]thymidine (New England Nuclear Corp.; 56.4 Ci/mmol) per ml. ^{32}P-labeled ϕ105 also was prepared by mitomycin C induction but using, in this case, VY medium that had been diluted 1:5 with water. $H_3{}^{32}PO_4$ (ICN Radiochemicals) was added to 10 μCi/ml at the time of induction. To prepare ^{14}C-labeled T7, *E. coli* O11′ was grown at 37°C in a medium consisting of Spizizen salts (29), 0.02% casein hydrolysate, 0.5% glucose, 50 μg of tryptophan per ml, 5 μg of thymidine per ml, and 0.25 μCi of [^{14}C]thymidine (New England Nuclear Corp.; 53.5 mCi/mmol) per ml. When the culture reached a density of approximately 10^8 cells/ml, the phage was added at a multiplicity of 1. After the culture had lysed, pancreatic DNase was added to 2.5 μg/ml and cellular debris was removed by centrifugation for 10 min at 12,000 × *g*. The phage was then pelleted by centrifugation for 60 min at 105,000 × *g*. The pelleted phage was resuspended in a 1.5-g/cm³ CsCl solution and centrifuged for 22 h at 33,000 rpm in a Spinco SW50 rotor. The collected phage band was dialyzed for 16 h against 0.01 M Tris-0.001 M EDTA, pH 8.0.

The radioactively labeled DNAs were isolated by phenol extraction as described above. The specific activities of ^{3}H-labeled ϕ105, ^{32}P-labeled ϕ105, and ^{14}C-labeled T7 DNA were 9,000, 80,000, and 4,100 cpm/μg, respectively.

DNA fragmentation by *Eco*RI. The reaction conditions used were those described by Polisky et al. (20). The 0.05-ml reaction mixtures contained 0.1 M Tris-hydrochloride (pH 7.4), 0.05 M NaCl, 0.005 M $MgCl_2$, 100 to 140 U of enzyme, and 1 to 2 μg of DNA. Incubation was at 37°C for 18 h, and the reaction was terminated by the addition of EDTA to 0.025 M followed by heating for 10 min at 65°C.

S1 nuclease. DNA fragments generated by *Eco*RI digestion were tested at 37°C with 5 μg of S1 nuclease in 0.05-ml reaction mixtures containing 0.1 M sodium acetate (pH 4.2), 0.2 M NaCl, 1×10^{-4} M $ZnCl_2$, and 1 to 2 μg of DNA (35). The nuclease had a specific activity of 57 U/mg, 1 U being the quantity of enzyme required to render acid soluble 1 A_{260}

(absorbance at 260 nm) unit of denatured DNA.

Ligation of φ105 DNA. A 1.2-μg amount of whole φ105 DNA was treated at 18°C for 24 h with 0.01 ml of *E. coli* ligase (25 U/ml) in a 0.05-ml reaction mixture (18). The reaction was terminated by heating at 65°C for 30 min, and 0.05 ml of the *Eco*RI buffer was added. The solution was then dialyzed for 24 h against 1,000 volumes of the buffer. The ligated DNA was digested with *Eco*RI as described above.

Agarose gel electrophoresis. Samples of 0.025 ml were analyzed on 0.7% agarose gels made up in a Tris-acetate buffer containing ethidium bromide (27). The digested DNA from the ligase reaction was electrophoresed in a horizontal gel apparatus at 40 mA for 24 h. All other gels were electrophoresed for 2.5 h at 90 V (9 V/cm) in a vertical apparatus. The separated DNA bands were visualized with short-wave UV light and were photographed through a red Wratten gelatin filter no. 25A, using type 57 Polaroid film.

Marker rescue. The procedure followed was that described by Armentrout and Rutberg (1). The φ105 DNA preparations used were unfractionated *Eco*RI-digested DNA and separated *Eco*RI-generated fragments that were recovered from the agarose gels by the "freeze-squeeze" method of Thuring et al. (32). *B. subtilis* strain GB7044 was grown to competence and exposed to DNA concentrations equivalent to 6 μg of intact φ105 DNA per ml for 20 min at 37°C. They were then superinfected at a multiplicity of approximately 5 with either φ105*sus*9 or φ105*sus*14, and the resulting phage was titered on su⁻ cells.

Sucrose gradient sedimentations. All centrifugations were performed at 20°C in a Spinco SW50.1 rotor at 44,000 rpm. The neutral 5 to 20% sucrose gradients contained 0.1% sodium dodecyl sulfate and 0.001 M NaCl; the alkaline sucrose gradients contained 0.9 M NaCl and 0.1 M NaOH. Molecular weights were determined from the neutral gradients by the method of Burgi and Hershey (5) and from the alkaline gradients using the equation developed by Studier (30).

RESULTS

***Eco*RI fragments.** Digestion of φ105 DNA with *Eco*RI generated eight fragments that were resolved by agarose gel electrophoresis (Fig. 1). The sum of the molecular weights of these fragments (Table 1), calculated from their mobility relative to *Eco*RI fragments of λ (31) and φ29 (16) DNAs, exceeded the 24 × 10^6 to 26 × 10^6 value determined to be the molecular weight of φ105 DNA by band sedimentation and electron microscopy (3, 7). As will be shown below, this discrepancy was due to the fact that fragment A was formed by the association of the cohesive ends of the molecule that are located on fragments C and D.

Identification of the cohesive end fragments. There are several lines of evidence indicating that fragment A consists of fragments C and D linked together.

Quantitation of the amount of DNA present in the separated *Eco*RI-generated fragments revealed that fragments A, C, and D were present in less than molar-equivalent amounts relative to the other fragments. The first indication of this, at least for fragments C and D, is evident in the gel shown in Fig. 1. In this gel, the ethidium bromide-stained bands C and D fluoresced with less intensity than band E. If each fragment in the digest is derived in a 1:1 ratio from the intact molecule, then the intensity of a fragment's fluorescence should be proportional to its molecular weight. This relationship was examined in greater detail, using ^{32}P-labeled DNA. The amount of radioactivity in the *Eco*RI-generated fragments was determined by counting 1-mm strips cut from a dried agarose gel and the logarithm of the counts per minute present in each fragment plotted against its

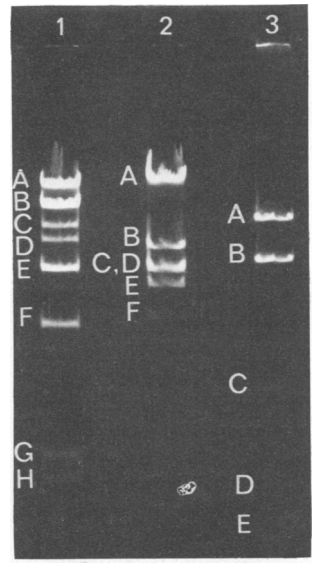

FIG. 1. *Electrophoresis of EcoRI-generated DNA fragments in 0.7% agarose gels. Slot 1, φ105 DNA; slot 2, λ DNA; slot 3, φ29 DNA. The samples were electrophoresed at 90 V (9 V/cm) for 2.5 h at room temperature in a vertical gel.*

TABLE 1. *Molecular weights of φ105 DNA fragments produced by cleavage with EcoRI endonuclease*[a]

Fragment	Mol wt (×10^{-6})[a]
A	11.0
B	7.1
C	5.8
D	5.2
E	3.5
F	2.1
G	0.6
H	0.4

[a] Values were calculated from relative electrophoretic mobilities, using the *Eco*RI fragments of λ (31) and φ29 (16) DNAs as standards.

electrophoretic mobility. Such a plot should produce a linear relationship between fragments present in equimolar amounts, and this was seen for fragments B, E, F, G, and H (Fig. 2); however, fragments C, D, and A deviated from the expected relationship.

Fragment A could be cleaved into two fragments migrating with the electrophoretic mobility of C and D by treatment with nuclease S1 (Fig. 3). The concomitant increase in the amounts of DNA at the positions of bands C and D, after S1 cleavage, was detected as an increase in the intensity of fluorescence of these bands. S1 nuclease has a pH optimum between 4.0 and 4.3 and is specific for non-base-paired or single-stranded DNA (35). Conversely, pretreatment of ϕ105 DNA with *E. coli* DNA ligase before *Eco*RI digestion decreased the amounts of fragments C and D produced (Fig. 4). In contrast to the above, treatment of the *Eco*RI digests at 45°C for 60 min with Pronase at 400 μg/ml or proteinase K at 500 μg/ml in the presence and absence of sodium dodecyl sulfate had no effect on the migration pattern (data not shown).

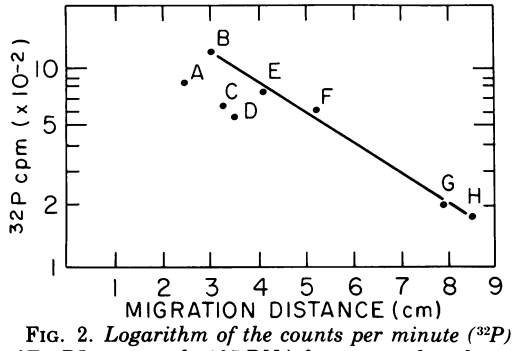

FIG. 2. *Logarithm of the counts per minute (^{32}P) of EcoRI-generated ϕ105 DNA fragments plotted as a function of electrophoretic mobility in a 0.7% agarose gel.*

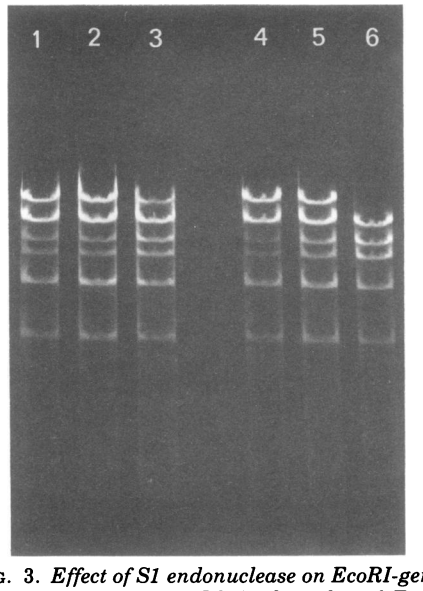

FIG. 3. *Effect of S1 endonuclease on EcoRI-generated fragments of ϕ105 DNA. Samples of EcoRI-cleaved DNA were dialyzed against 0.1 M sodium acetate-0.2 M NaCl-0.1 mM $ZnCl_2$ at the pH values indicated below. Portions were then treated with S1 nuclease for 60 min at 37°C and electrophoresed as described in Fig. 1. Slots 1, 2, and 3 contain samples dialyzed at pH 6.0, 5.0, and 4.2, respectively, but not treated with S1 nuclease. Slots 4, 5, and 6 contain samples dialyzed at pH 6.0, 5.0, and 4.2, respectively, followed by treatment with S1 nuclease.*

Origin of fragments C and D. Fragments C and D were isolated from agarose gels as described under Materials and Methods and tested for the presence of end-proximal genes by marker rescue. The phage mutants used in these experiments carried either a *sus*14 or *sus*9 mutation. *sus*14 maps in gene *B*, and *sus*9 maps in gene *L*; the former is near the left end of the genetic map, and the latter is at the right end (1, 24). The results of these experiments (Table 2) indicate that fragment C is derived

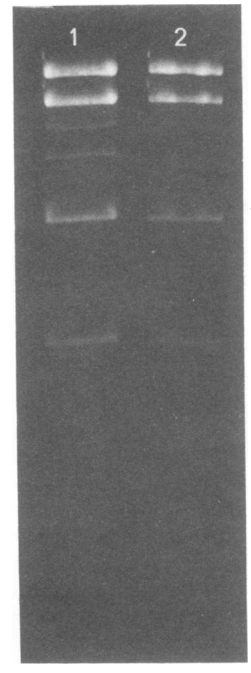

FIG. 4. *Sealing of the associated end fragments of ϕ105 DNA by E. coli DNA ligase. Untreated and ligase-treated samples of ϕ105 DNA were digested with EcoRI endonuclease and then subjected to agarose gel electrophoresis at 40 mA for 24 h in a horizontal gel apparatus. Slot 1, EcoRI fragments derived from untreated DNA; slot 2, EcoRI fragments prepared after treatment of the intact DNA with DNA ligase.*

from the right and fragment D from the left end of the genome. The unfractionated *Eco*RI-digested DNA rescued both the *sus*9 and *sus*14 mutations at a similar efficiency. With the isolated fragments, the *sus*9 mutation was rescued at a high efficiency by fragment C and the *sus*14 mutation was rescued at a high efficiency by fragment D. Both fragment preparations exhibited a lower level of rescue activity with the marker from the other end of the chromosome. The relative rescue efficiency of *sus*14 by the fragment C preparation was 8% that observed for *sus*9 with the same DNA, and the relative rescue efficiency of *sus*9 by the fragment D preparation was 3% that observed with this preparation for *sus*14. This low level of rescue was probably due to cross-contamination of the fragment preparations. Fragments C and D migrated close to each other, and, in fact, when the isolated fragments were re-electrophoresed, low levels of cross-contamination could be observed.

Sedimentation characteristics of intact and *Eco*RI-digested φ105 DNA. It is evident that a large percentage of the end fragments of *Eco*RI-digested φ105 DNA migrate as joined molecules in agarose gels. Since *Eco*RI digestion involves a prolonged incubation at 37°C, a condition that might lead to either circle or oligomer formation, it was of interest to examine the structure of freshly isolated DNA. For this purpose, ^{3}H-labeled φ105 DNA was cosedimented in neutral and alkaline sucrose gradients with ^{14}C-labeled T7 DNA, a linear molecule with the same molecular weight as φ105 DNA.

In neutral gradients, nearly all of the undigested φ105 DNA sedimented ahead of the position expected for the linear monomer (Fig. 5A). The majority of the material formed a peak at the position expected for an open circular structure, with the remainder sedimenting as circular or linear oligomers (34). The end-joined fragments in the *Eco*RI digest (Fig. 5C) were also detected in the neutral gradients appearing as a shoulder on the heavy side of the main peak of DNA. The molecular weight of the material in this shoulder was calculated to be 10.8×10^6, which is in good agreement with the 11×10^6 value expected for the joined C and D fragments.

In the alkaline gradients, whole φ105 DNA co-migrated with the T7 marker (Fig. 5B), and the *Eco*RI-digested material formed a broad band covering an approximate molecular-weight range of 1.1×10^6 to 4.7×10^6 (Fig. 5D). No evidence for covalently closed circles or covalently linked oligomers was found.

TABLE 2. *Marker rescue of end-proximal genes from EcoRI fragments C and D of φ105 DNA*[a]

DNA	Infectious centers	
	*sus*14	*sus*9
*Eco*RI digested	2.4×10^4	3.5×10^4
Fragment C	0.7×10^3	8.6×10^3
Fragment D	3.6×10^3	0.1×10^3

[a] Competent su⁻ bacteria were incubated at 37°C with DNA digested with *Eco*RI or with purified *Eco*RI fragments C and D. The DNAs were added at concentrations equivalent to 6 μg of intact φ105 DNA per ml and 20 min later the cells were superinfected, at a multiplicity of 5, with phage carrying the *sus* markers indicated above. After 15 min of further incubation, the infected cells were assayed for PFU on an su⁻ indicator strain (1). The background levels of infectious centers produced by the *sus* phages in the absence of added DNA (2×10^2 PFU/ml) were subtracted from the above values.

DISCUSSION

It had previously been shown that φ105 DNA is made up of a unique, not circularly permuted nucleotide sequence and that the DNA has co-

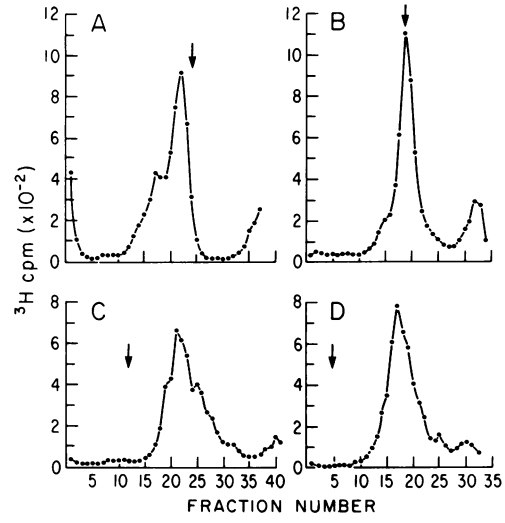

FIG. 5. *Neutral and alkaline sucrose gradient sedimentation of untreated and EcoRI endonuclease-treated ^{3}H-labeled φ105 DNA. Each gradient contained 0.5 μg of ^{3}H-labeled φ105 DNA in the buffer used for the EcoRI endonuclease digestions. The samples were layered onto 5 to 20% neutral and alkaline sucrose gradients and centrifuged as described in the text. The fractions collected were counted in Aquasol (New England Nuclear Corp.) using a Packard Tri-Carb liquid scintillation counter. (A) Untreated φ105 DNA sedimented in a neutral sucrose gradient; (B) untreated φ105 DNA sedimented in an alkaline sucrose gradient; (C) EcoRI-digested φ105 DNA sedimented in a neutral sucrose gradient; (D) EcoRI-digested φ105 DNA sedimented in an alkaline sucrose gradient. ^{14}C-labeled T7 DNA was added to each gradient as a marker. The position of the marker DNA is indicated by the arrow. Sedimentation was from right to left.*

hesive ends (1-3, 7). The results presented here indicate that ϕ105 DNA, as isolated from the phage particle, consists primarily of circular molecules and contains six *Eco*RI cleavage sites. The largest fragment separated by agarose gel electrophoresis of the *Eco*RI-digested DNA is formed by the noncovalent association of the two smaller fragments, C and D. Fragment C was shown, by marker rescue, to be derived from the right end of the linear form of the molecule and fragment D from the left end. It should be noted that a somewhat different *Eco*RI cleavage pattern of ϕ105 DNA has been reported by Wilson et al. (36). Their digestions, however, were done in a low-ionic-strength buffer, and the substrate specificity of *Eco*RI was reduced in buffers of low ionic strength (20).

It seems likely that cyclization of ϕ105 DNA occurs through the association of complementary single-stranded nucleotide sequences at the ends of the molecule. This mechanism has previously been suggested by Chow et al. (7) on the basis of electron microscopic studies of denatured-renatured molecules. It is supported by the effects of both *E. coli* ligase and S1 nuclease on the associated ends of ϕ105 DNA. Studies of the substrate specificity of *E. coli* ligase have shown that it can join molecules annealed through single-stranded cohesive ends, but unlike phage T4 ligase it fails to join fully base-paired termini (25). Cleavage of the associated end fragments by S1 nuclease probably occurs at the junction points (10) of the cohesive ends. These sites would appear essentially as nicks in a double-stranded DNA, and as such would be substrates for this nuclease (28). No evidence was found for the involvement of a protein in the association of the end fragments. Treatment of the *Eco*RI-digested DNA with either proteinase K or Pronase did not alter the migration pattern of the fragments. Also, protein-DNA complexes of the sort seen with ϕ29 and adenovirus do not migrate very well in the agarose gels (16; R. Roberts, personal communication), and no significant amounts of ϕ105 DNA are observed remaining at the origin after electrophoresis as can be seen with ϕ29 DNA (16).

The cohesive ends of ϕ105 DNA appeared to associate with each other more readily than those of λ. Considerable amounts of end-joined fragments were detectable in the agarose gels of ϕ105 *Eco*RI digests even though this material had been heated to 65°C for 10 min and quenched in an ice bath immediately before electrophoresis. Since this treatment disrupts the associated ends of λ DNA (15), which are more stable to denaturing conditions than those of ϕ105 (7), it seems likely that the ϕ105 ends rapidly reassociate once the denaturing conditions are removed. It has been reported that the activation energy required for the association of λ DNA ends is higher than expected for renaturation, and it has been suggested that the λ ends contain a secondary structure that must be disrupted for end-to-end association to occur (10).

The fact that the predominant form of ϕ105 DNA is a circle may explain a number of earlier observations such as the effects of annealing conditions (3) and exonucleases (2) on the native molecule. Attempts to alter the sedimentation characteristics of ϕ105 DNA by subjecting preparations to conditions favoring the formation of circular and oligomeric forms by λ DNA failed, presumably because the molecules were already circular. The resistance of ϕ105 DNA to degradation by *E. coli* exonuclease I is consistent with the inability of this enzyme to act at a nick in fully duplexed DNA (4). ϕ105 DNA is degraded by *E. coli* exonuclease III but at a much slower rate than λ DNA (2). This enzyme does initiate degradation on nicked strands, but the affinity of the enzyme for nicked structures is less than its affinity for free ends (17).

With respect to the probable mode of ϕ105 integration, assuming that the predominance of circular structures seen in vitro also occurs in infected cells favors a site-specific recombination mechanism, with the *att* site either formed by or situated close to the joined ends. Integration through a single *att* site on a circular molecule, in contrast to an integration involving two separate *att* sites, would not cause a deletion of bacterial DNA. The single *att* site mode of integration also has been suggested by recent genetic studies showing that lysogenization by ϕ105 decreases the linkage between markers on either side of the ϕ105 insertion site by an amount expected for the insertion of 1 to 2 ϕ105 genome lengths of DNA (11). It should be noted, however, that deletions corresponding to some fractional length of ϕ105 DNA probably would not be detected by this type of study.

Finally, we would like to comment on the use of ϕ105 DNA as a substrate for assaying DNA ligase activity. Disappearance of the *Eco*RI-generated end fragments of λ DNA has been suggested as a rapid method of assaying DNA ligases during their isolation and purification (19). In the case of λ, however, the end fragments are fragments A and F, that is, the largest and smallest of the λ fragments, and ligation of fragment F to A does not significantly alter the latter's mobility on 0.7 or 1.0% agarose gels. Ligation of the ϕ105 end fragments, on the other hand, clearly alters the electrophoretic mobility of both fragments. This may prove important in purifying ligases from crude ex-

tracts that may contain nucleases capable of rapidly degrading the smaller DNA fragments.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grants GM21541 from the National Institute of General Medical Sciences and NCI 5 P30 CA-16058 from the National Cancer Institute.

We gratefully acknowledge the expert technical assistance of V. Lima.

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