Deletion Mutants of Bacteriophage φX174

(electron microscopy/DNA heteroduplex/density gradient centrifugation/polyacrylamide gel electrophoresis)

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ABSTRACT Mutants of bacteriophage φX174 have been isolated that are less dense than wild-type φX particles in CsCl. When mutant viral (+) strand DNA and wild-type complementary (−) strand DNA are hybridized, the resulting duplex molecules have single-stranded loops characteristic of wild-type–deletion heteroduplexes. The mutant bacteriophages fail to complement φX amber mutants in cistron *E*. We conclude that the mutant viruses have deleted approximately 7% of the φX genome in the region of cistron *E*.

Genetically characterized deletions have been used as physical markers in the heteroduplex mapping of the genomes of bacteriophages λ, T4, and φ80 by electron microscopy (1–4). The loss of a region of DNA 100 nucleotides in length is detected as a single-strand loop with this technique (5). Genetically defined deletions of 100 or more nucleotides of bacteriophage φX174 DNA characterized in this way would be valuable tools for determining the locations of such structural features of the φX174 DNA molecule as the specific nick in the *in vivo* replicative form (6), the initiation sites of complementary strand and viral DNA synthesis, the non-homologous regions in heteroduplexes with DNA from φX-like phages (S13, ST-1, α-3, and the binding sites of *Escherichia coli* RNA polymerase. The shorter contour length of the deleted DNA molecule as measured by electron microscopy distinguishes it from normal-length molecules in genetic experiments involving mixed infections (7). In addition, deletion mutants may provide a means for generating unique fragments of φX DNA for nucleotide sequencing, and hetero-duplex deletion loops may be used as reference markers to map φX DNA fragments produced by other methods.

Since the genome of φX174 has been extensively mapped (8), and recombination frequencies are proportional to physical distances over much of the genetic map (9), a correlation could be made between the physical location of each structural feature and the known φX cistrons.

A deletion is a nonsuppressible genetic defect; such mutants are viable only if the lesion is limited to dispensable viral functions. Of the nine known φX174 cistrons (8) only *E* is not essential to the production of infective viral particles (10, 11). Mutants in cistron *E* are impaired in the lysis function (10). They produce large numbers of apparently normal virions, which remain trapped within the walls of an intact nonsuppressing bacterial host cell (10). We have exploited

this property to enrich for deletions in the cistron *E* region of the genome. Further selection, based upon the buoyant density of mature virus particles, was done to obtain mutants that had lost an appreciable portion of the cistron. We report here some of the physical and genetic characteristics of a virus stock obtained by this means.

MATERIALS AND METHODS

Media. KC broth (12), plating agar (13), and TPG (14) have been described. TPA medium was made by addition of 0.3 g of each of 20 individual L-amino acids to 1 liter of TPG medium. TPA-Leu is TPA medium from which the amino acid L-leucine was omitted. Borate dilution buffer contains 0.05 M sodium tetraborate, pH 8.0. Borate–EDTA contains 0.05 M sodium tetraborate–5 mM EDTA.

L-[^{14}C]Leucine (316 Ci/mol), L-[4,5-^{3}H]leucine (2 Ci/mmol), [2-^{14}C]thymidine (50 Ci/mol), and [methyl-^{3}H]thymidine (10 Ci/mmol) were purchased from Schwarz/Mann.

Rabbit antiserum to φX174 was prepared by R. G. Rohwer, by the procedure of Rolfe and Sinsheimer (15).

Bacterial and Virus Strains. *Escherichia coli* C, BTCC No. 122 (12) is our standard nonpermissive host for φX174.

H502 is a *thy⁻*, *hcr⁻*, *endo* I⁻ nonpermissive host strain that was constructed in the laboratory of Dr. Hoffman-Berling.

HF4714 is a permissive host strain that suppresses most amber (UAG) nonsense mutants of φX174 (8).

Su2$_{och}$ is an amber (UAG) and ochre (UAA) suppressor strain (16) with the genotype *thy⁻, cyt⁻, met⁻, pro⁻, trp⁻*.

CIT103 suppresses opal (UGA) nonsense mutants of φX174 and is *lac⁻* and streptomycin resistant (11).

wt is the φX174 wild-type virus characterized by Sinsheimer (12).

Virus Stocks. The preparation of genetically pure stocks of φX174 amber mutants was reported (8). [^{3}H]Leucine- and [^{3}H]thymidine-labeled particles of φX174 *wt* phage were prepared (9) with H502 host cells growing in TPA-Leu with 2 μg/ml of thymine. DNA was extracted from purified viruses by the procedure of Sinsheimer (17).

Isolation of φX wt Complementary (−) Strands. Double-stranded φX replicative form (RF) DNA was prepared according to Komano and Sinsheimer (18). RFI was isolated by exclusion chromatography on a Bio-Gel A 1.5-m column, followed by equilibrium buoyant density centrifugation in CsCl with 100 μg/ml of propidium iodide (CalBiochem).

Abbreviations: RF, replicative (double-stranded) form of φX174 DNA; RFI, replicative form DNA with both strands covalently closed; RFII, replicative form DNA with one or more discontinuities in either strand; MOI, multiplicity of infection.

RFI molecules were given an average of 1.7 nicks per duplex by subjecting them to x-rays generated by a General Electric industrial x-ray unit. Complementary strands were enriched from the resulting RFII molecules by equilibrium buoyant density centrifugation in CsCl (1.75 g/ml)–0.1 M KOH at 30,000 rpm in a Spinco Type 50 rotor (19).

Isolation of Deletion Mutants. Deletion mutants were generated by infection of a culture of *E. coli* C in the presence of 1 μg/ml of mitomycin C (Nutritional Biochemicals Corp.) with *wt* φX174 at a multiplicity of infection (MOI) of 10. Under these conditions any defective viruses produced in the presence of mitomycin C (20, 21) would be released into the medium by the action of the numerous *wt* virions also present in the cell. Phage concentrated from this lysate were used to infect a culture of HF4714 at 1×10^8 cells/ml at a low MOI [less than 0.2 *wt* plaque-forming units (PFU)/cell]. Under these conditions, unsuppressed lysis-defective viruses would remain within intact host cells.

EDTA was added 15 min after infection to remove calcium ions from the medium and thereby prevent secondary adsorption of newly released *wt* phage (22). After 90 min of incubation, the cells were harvested by centrifugation and washed five times with borate–EDTA to remove any free *wt* phage particles (22). The cells were disrupted by incubation for 30 min at 37° in 5 ml of 0.05 M borate–0.05 M EDTA–25 mM Tris (pH 8.0), containing 0.3 mg of lysozyme per ml. The lysate was treated for 30 sec with a Branson sonifier and the released phage were used to infect a second culture of HF4714 at low MOI (less than 0.2 *wt* PFU/cell). The cell washing procedure, lysis, and infection of a new culture at low MOI were repeated sequentially 15 times.

Lysates were monitored for PFU on *E. coli* C, HF4714, and Su2$_{och}$. The titers indicated that there was no significant accumulation of suppressible nonsense mutants during the procedure. Lysates subsequent to the fifteenth infection cycle were centrifuged to equilibrium in CsCl (0.61 g/ml) at 25,000 rpm in a Spinco SW41 rotor. The gradients were collected through a needle by puncture of the tube wall near the bottom. Selected fractions were diluted in borate, and the absorbance at 260 nm was read in a Zeiss spectrophotometer. Fractions from the "light" edge of the phage peak were pooled and used for propagation in the next cycle of infection. The recycling infection procedure was repeated an additional eight times with that modification.

Virus particles from the last round of infection were inoculated at a multiplicity of 8 phage/cell into a 200-ml culture of H502 at 4×10^8 cells/ml in TPA-Leu containing 2 μg/ml of thymine. After 5 min, 100 μCi of [¹⁴C]leucine or [2-¹⁴C]thymidine was added to the medium. After 2 hr of incubation, the cells were collected by centrifugation, washed twice with 200-ml volumes of borate–EDTA, and lysed with lysozyme. The phage were purified by equilibrium centrifugation in CsCl (0.61 g/ml)–borate–EDTA, followed by zone centrifugation in a linear 5–20% sucrose gradient in 0.05 M Tris–5 mM EDTA (pH 7.4). Alternatively, the isopycnic centrifugation in CsCl was replaced by exclusion chromatography on a 1 cm × 90 cm column of porous glass beads (23). These purified phage preparations will be called the "deletion stock."

Complementation Plaque Assay of Deletion Stock. "Helper phage" (specific amber mutants in any φX cistron) at a multiplicity of about 5 PFU/cell were adsorbed at 37° for 5 min in

2-ml aliquots of *E. coli* C at 1×10^8 cells/ml in KC broth containing 3 mM KCN. The suspension was divided in half, and a portion of the deletion stock was added to one of the two tubes. After both suspensions had been incubated at 37° with aeration for 15 min, 5 ml of an *E. coli* culture at 4° containing about 5×10^8 cells/ml in KC broth with 3 mM KCN was added as carrier, and the tubes were centrifuged at 4° at 6000 rpm for 15 min. The pellets were washed twice in 5-ml volumes of KC broth with cyanide, and were resuspended in 1.0 ml of KC broth with cyanide and antiserum to φX (K = 25/min). After incubation for 10 min at 37°, the suspensions were diluted 1:10⁴ in warm KC broth without cyanide, and the tubes were transferred to an ice bath. Aliquots were plated by the agar layer technique with HF4714 plating bacteria.

Electron Microscopy of DNA Heteroduplexes. Heteroduplex DNA molecules (1, 2) containing deletion stock single-strand viral (+) DNA and *wt* complementary (−) strand DNA were constructed (24). Specimens were photographed at ×17,500 under a Philips EM300 electron microscope. Negatives were enlarged ×20 on a Nikon shadowgraph, traced, and measured with a Keuffel and Esser map measurer.

Polyacrylamide Gel Electrophoresis. 15% Polyacrylamide gels containing 0.375 M Tris (pH 8.0)–0.1% sodium dodecyl sulfate were used (9). Gels (9.0 cm long) were polymerized in Pyrex glass tubes with an inner diameter of 7.0 mm. Mixtures of [¹⁴C]- and [³H]leucine-labeled phage particles were disrupted by boiling in 8 M urea for 10 min immediately before electrophoresis. (9). The gels were sliced with a Mickle Gel-Slicer (Brinkmann Instruments) and counted in 5 ml of scintillation fluid [858 ml toluene, 90 ml NCS (Amersham/Searle), 42 ml Liquifluor, and 10 ml of distilled water].

RESULTS

Equilibrium Centrifugation in CsCl. The "deletion stock" virus particles band at a lower buoyant density in CsCl than does *wt* φX174 (Fig. 1). Based upon refractometry of several fractions from the gradients, the mutant particles have a density of 1.4035 g/cm³ compared to 1.4079 g/cm³ obtained for *wt* phage, an average density difference of 0.0044 ± 0.0003 g/cm³ in four determinations. This characteristic difference was retained after phage from the "deletion stock" were propagated and purified (without selection on the basis of buoyant density) by chromatography on a porous glass bead column or when φX cistron *E* mutant *am3* phage particles were substituted for *wt* φX phage particles.

This result suggests that the DNA–protein ratio of the deletion stock virus is different from that of *wt* or *am3* viruses. Such an alteration may be explained either by a reduction in the molecular weight of the DNA in these virions or by an increase in the quantity of protein in the purified mutant particle.

Sedimentation Velocity of Deletion Particles in Sucrose Gradient. For investigation of the latter possibility, ¹⁴C-labeled deletion stock phage were mixed with ³H-labeled *wt* particles and centrifuged in neutral sucrose gradients. Fig. 2 shows that the two viruses sediment together under these conditions, indicating that the hydrodynamic properties of the mutant virus are not detectably different from those of the *wt* virus.

Polyacrylamide Gel Electrophoresis of Bacteriophage Particles. We have examined the coat proteins of the deletion stock
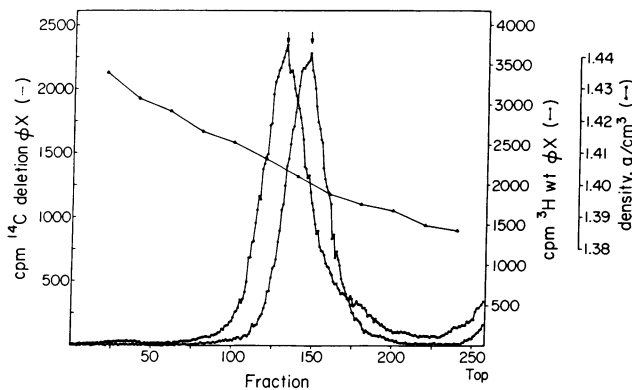
FIG. 1.   Equilibrium buoyant density centrifugation of purified deletion and wt φX174 phage particles. [14C]Thymidine-labeled deletion phage (O——O) and [3H]thymidine wt φX phage (●——●) were mixed in 8.0 ml of solution containing CsCl (ρ = 1.400 g/cm³)–0.05 M borate–5 mM EDTA (pH 8.0). The material was centrifuged for 60 hr at 25,000 rpm in a Spinco Type 50, fixed-angle rotor at 5°. Fractions were collected directly into scintillation vials containing 10 ml of Aquasol. Several fractions throughout the gradient were collected into a small quantity of oil, and their density was determined by refractometry (▲——▲).

FIG. 2.   Sedimentation of purified deletion and wt φX174 phage particles in a neutral sucrose gradient. [14C]Leucine-labeled deletion phage and [3H]leucine wt phage were mixed and layered onto a 13.0 ml, linear 5–20% sucrose gradient containing 0.05 M Tris (pH 8.1)–5 mM EDTA. The preparation was centrifuged at 24,000 rpm for 6.5 hr in a Spinco SW40 rotor at 5°. Fractions were collected into scintillation vials, which contained 10 ml of Aquasol.

viruses by disc electrophoresis in polyacrylamide gels. [3H]-Leucine-labeled wt particles and [14C]leucine-labeled deletion particles were mixed, disrupted with urea, and applied to 15% gels. The 14C- and 3H-specific counts in each slice of one such gel after electrophoresis are shown in Fig. 3. The deletion phage particles contain six electrophoretic components identical to those of the wt virus. Four of the proteins correspond to the products of cistrons F, H, G, and J identified by Benbow et al. (9), in spite of the fact that their relative mobilities (R_F) are somewhat greater than those previously reported (9, 25). The small peak (R_F = 0.56) appearing as a shoulder on the leading edge of the H protein (R_F = 0.54) is presumed to be the product of cistron B, which has been identified as a minor phage component (9, 26). The peak at R_F = 0.75 has not been previously observed in φX virions, and may be a contaminant. However, its appearance as a constant fraction of the counts in both preparations, while there is a considerable variation in the small amount of radioactivity found in regions of the gel presumed to be lacking phage proteins, suggests that it is not a chance impurity.

The percentages of the total radioactivity found in each peak are shown in Table 1. With the exception of the cistron H product, the relative amounts of the remaining five proteins in the deletion stock particles do not differ significantly from those of the wt virus. The pattern of deletion phage proteins including the H product is nearly identical to that seen in the reported (9, 27) particles of cistron E mutants when the R_F = 0.75 component is added to the G peak (Table 1).

Heteroduplex Mapping in the Electron Microscope.   In order to determine if the mutant and wt viruses contain the same nucleic acid moiety, we have constructed heteroduplex molecules containing one strand of DNA from each source. Examination of these preparations by electron microscopy revealed many double-stranded molecules with a small, single-stranded loop (Fig. 4) characteristic of a deletion–wt heteroduplex. 74 Such molecules on a single grid were photographed and measured. All the single-stranded circular molecules
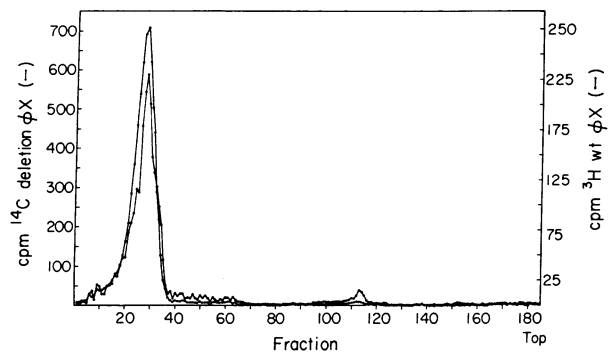
present in these photographs were also traced and measured. Fig. 5 shows that the contour lengths of the single-stranded circles are distributed in two modes, representing the wt and mutant DNA molecules in the hybridization mixture. The peak of the shorter mode differs from the larger by 6.8%. The average length of the single-strand loops in the duplex molecules is 0.082 ± 0.018 of the wt φX genome (the larger mode in the distribution of single-strand circles in Fig. 5).

The Genetic Defect of the Deletion Virus.   The purified deletion phage stock contains 1.18 A_{260nm} units/ml, or a calculated 1.52 × 10¹⁴ phage particles/ml (12). The deletion stock phage, however, do not form plaques efficiently on amber, ochre, or opal suppressor host strains under the conditions used to plate φX mutants (Table 2). Phage stocks not carrying deletions that have been subjected to the same purification procedure gen-
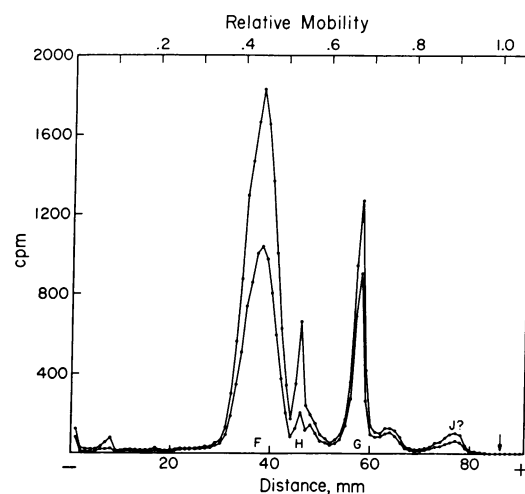

FIG. 3.   Electrophoresis of purified deletion and wt φX174 phage particles in polyacrylamide gels. [14C]Leucine-labeled deletion phage (O——O) and [3H]leucine wt phage particles (●——●) were mixed, disrupted with urea, and applied to 15% polyacrylamide gels. The gel was cut into 1.0-mm slices, and each was counted in NCS scintillation fluid. The arrow indicates the position of the bromphenol blue tracking dye.

TABLE 1. *Percent of total radioactivity in electrophoretic components of φX174 phage particles**

	Cistron Observed Mobility (R_F)	F 0.43 (0.37)†	H 0.54 (0.50)†	B? 0.56 (0.59)†	G 0.67 (0.63)†	— 0.75 —	J? 0.90 (0.82)†
wt		64.5 ± 0.5	4.0 ± 0.3	3.9 ± 0.3	21.0 ± 0.3 (24.8)‡	3.8 ± 0.2	2.8 ± 0.1
Deletion		65.9 ± 0.3	6.7 ± 0.1	3.4 ± 0.1	17.9 ± 0.1 (21.2)‡	3.3 ± 0.2	2.8 ± 0.1
am27† (E)		65.4	6.6	1.4	22.9	—	3.7
am3§ (E)		66.7 ± 0.4	7.6 ± 1.2	—	20.2 ± 1.2	—	5.5 ± 0.4

* Numbers are averages of four gels, with standard deviation from the mean.

† Benbow *et al.* (9); labeled with [³H]leucine.

‡ Sum of $R_F = 0.67$ and $R_F = 0.75$ components.

§ Burgess (27); labeled with 14 [³H]aminoacid mixture.

erally have specific infectivities of 0.05–0.5 PFU/particle (12, 22), more than 100 times higher than that observed with the deletion stock.

In order to identify the φX cistrons that have been affected by the genetic lesion in the deletion phage DNA, we have modified the method used to detect complementation between mutants of φX174 (11). In addition, this technique serves as a quantitative plaque assay for the deletion phages. Under the conditions we use, φX deletion mutants will form plaques if the "helper phage" has a functional cistron at the position corresponding to the genetic defect of the deletion. A plaque is formed when a multiply-infected nonsuppressing host cell lyses and releases "helper phage" particles that are able to attack the amber suppressor host cells in the lawn.

Table 3 shows the plaque-forming ability of the deletion stock with "helper phage" having mutations in each of seven φX cistrons. "Helper phage" with amber mutations in all of the cistrons except E increased the plaque-forming ability of the deletion stock from 4- to 200-fold. The deletion phage titers obtained with am3 and am27, mutants in cistron E, were identical to those observed when the deletion phage were plated on *E. coli* C without any "helper."

DISCUSSION

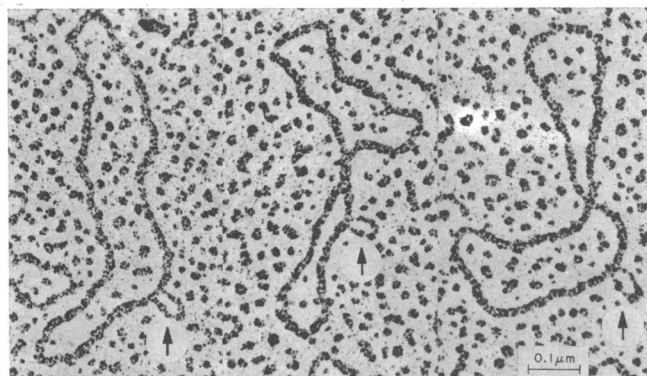The data presented above suggest that we have isolated a collection of deletion mutants of bacteriophage φX174 that

FIG. 4. Electron micrographs of heteroduplex DNA molecules produced by hybridization of deletion viral (+) strands with wt φX174 complementary (−) strands. Image magnification is ×66,600. *Arrows* indicate the single-stranded loops, which locate the deleted regions.

lack a portion of cistron E, the gene that controls the host lysis function.

The bimodal distribution of contour lengths (Fig. 5) obtained when wt and mutant stock single-strand DNA are examined in the same field in an electron microscope is strong evidence that the mutant virus genome is shorter than that of wt. The appearance and the lengths of the single-stranded loops in heteroduplexes of wt and mutant DNA (Figs. 4 and 5) confirm this conclusion and indicate that the lesion in each DNA molecule is localized and not widely distributed over the genome. Indeed, the deletions are likely to be confined to cistron E, since the mutant viruses fail to complement amber mutants in that gene but are able to supply the defective functions of amber mutants in six other cistrons (Table 3).

We have attributed the change in buoyant density of the mutant virus particles (Fig. 1) to their loss of DNA. The sedimentation velocity of these viruses (Fig. 2) and the electrophoretic pattern of the viral proteins (Fig. 3 and Table 1) indicate that the protein structure of the mutant viruses is not sufficiently different from wt or am3 to account for the observed 0.0044 g/cm³ decrease in particle density. Accordingly, we applied the relationship between increment in density and change in DNA content formulated by Weigle, Meselson, and Paigen (30) for bacteriophage λ

$$\Delta\rho = \rho_0\alpha \frac{F_m - F_v}{1 + \alpha F_v} \quad *$$

to obtain a value of 6.3% for the percent of φX wt DNA deleted. This figure agrees very well with the estimate of deletion length obtained from contour measurements of single-stranded DNA (6.8%). The size of the deleted region calculated from the single-strand loops seen in heteroduplex molecules (8.2%) may be biased by the tendency to select molecules with large, obvious deletion loops. Nevertheless, it agrees within experimental error with the first two estimates.

Considering the manner in which the deletion viruses were selected and the fact that unsuppressed mutants in cistrons other than E are not expected to produce infectious phage

* The values for wt φX174 used in this calculation are 1.408 g/cm³ for the phage buoyant density (ρ_0), 0.269 for the mass fraction of DNA in the phage (F_m) (12), and 0.220 for the volume fraction [F_v, the mass fraction, multiplied by the ratio of the phage buoyant density, 1.408 g/cm³ to the DNA buoyant density, 1.725 g/cm³ (31)].

TABLE 2. *Plaque-forming ability of deletion and wt phages on suppressor host strains*

Host strain	Suppressor	Deletion stock		wt φX174*	
		PFU/ ml ×10⁻¹⁰	PFU/ particle† (×10³)	PFU/ ml ×10⁻¹⁰	PFU/ particle†
C	su⁻	2.3	0.15	9.3	0.13
HF4714	UAG	7.4	0.49	8.4	0.12
Su2*och*	UAG, UAA	7.8	0.51	6.6	0.092
CIT103	UGA	1.3	0.086	12.0	0.17

* Purified by the same method as the deletion stock.

† 1.20×10^{13} phage particles/A_{260nm} unit in purified *wt* preparations (12), corrected to $1.28 \times 10^{13}/A_{260nm}$ for particles with a 7% deletion of DNA.

TABLE 3. *Plaque-forming ability of deletion stock with φX174 amber mutants as helper phage*

Helper phage	Mutant cistron	Deletion stock PFU/ml	Relative number of PFU
None	—	2.3×10^{10}	1.0
*am*10*	D	4.5×10^{12}	200
*am*3*	E	$<2 \times 10^{10}$	<1
*am*27*	E	2.8×10^{10}	1.2
*am*88†	F	5.2×10^{11}	23
*am*87†	F	5.3×10^{11}	23
*am*9*	G	1.3×10^{12}	57
*am*N1‡	H	2.0×10^{12}	87
*am*86†	A	9.0×10^{10}§	3.9
*am*14*	B	1.6×10^{12}	70

* Isolated by Dr. C. A. Hutchison (11).

† Isolated by Dr. F. Funk (28).

‡ Obtained from Dr. M. Hayashi.

§ This low value is probably a consequence of the asymmetric complementation observed for mutants of cistron *A* (29).

under these conditions, all of the observed deletions in the mutant stock are probably confined to gene *E*. Benbow *et al.* (9) report that the presumed gene *E* product has a molecular weight of 17,500, representing 7.7% of the total protein coding capacity of a φX genome with 5500 nucleotides. This figure might be considered the upper limit for the length of the deleted region in members of the mutant stock.

In our laboratory, *am*3, the cistron *E* mutant of φX174, is routinely used as a substitute for *wt* φX because its lysis defect facilitates the preparation of phage stocks of high titer (10). In some cases, this mutant has been grown for many generations under nonpermissive conditions without cloning. After each propagation the cells were lysed artificially to release the phage particles. Such conditions permit the transmission of deletions in cistron *E* that may arise spontaneously. Indeed, the elimination of the cistron may confer an advantage upon the mutant by permitting it to replicate more quickly than its *am*3 ancestor.

In a companion paper, Kim, Sharp, and Davidson (32) report their discovery of partially deleted φX DNA molecules that appeared spontaneously in samples of *am*3 RF grown under such conditions in our laboratory. We have denatured a portion of their "sample 1" and renatured it in the presence of viral DNA from the deletion stock described in this paper.

Nearly all the molecules were perfect duplexes or had a single deletion loop. A few molecules were observed with one small single-strand loop, indicating some heterogeneity in the extent of the deletions in one or both of the component DNA stocks. We believe that the length of the deleted region varies in members of our mutant virus population. This is reflected, to some degree, in the asymmetric shape of the deletion loop histogram in Fig. 5. Apparently, many members of the stock have deleted the entire cistron, but others have lost smaller segments.

Benbow, Eisenberg, and Sinsheimer (7) measured the contours of single- and multiple-length DNA molecules in the RF preparation that Kim *et al.* (32) have called "sample 2." The contours of the multiple-length molecules are exact multiples of either *wt* φX length or of the deletion DNA length. This observation suggests that the deletion DNA replicates *in vivo*, and that deletion viral strands are not generated by premature termination of replication from a *wt* RF molecule.

In summary, our results indicate that we have isolated a population of deletion mutations of bacteriophage φX174 that are (*i*) genetically defined within the cistron *E* region, (*ii*) physically defined as individual deletions with an average size of 7% of the φX genome (about 390 nucleotides), (*iii*) able to propagate as efficiently as φX174 *wt* or *am*3, and (*iv*) possess an essentially normal viral coat structure and all viral functions except host cell lysis.

These mutants, which we shall call φX174 *del*E, represent the first reported genetically and physically characterized deletion mutants of a small DNA bacteriophage.
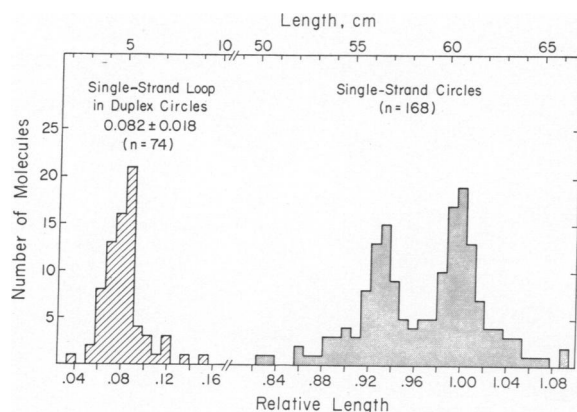
FIG. 5. Length distributions of single-stranded loops in heteroduplex DNA molecules and single-stranded circular DNA molecules in a partially renatured mixture of deletion viral (+) strands and *wt* φX174 complementary (−) strands. All measurements were made from photomicrographs of a single grid.

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