

# Infection by Cholera phage $\phi$ 138: Bacteriophage DNA and Replicative Intermediates

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**Cholera phage  $\phi$ 138 contains a linear, double-stranded, circularly permuted DNA molecule of  $30 \times 10^6$  daltons or 45 kilobase pairs. Upon infection, the host DNA is degraded, and synthesis of phage-specific DNA is detectable 20 min after infection. The phage utilizes primarily the host DNA degradation products for its own DNA synthesis. A physical map of  $\phi$ 138 DNA was constructed with the restriction endonucleases *Bg*III, *Hind*III, and *Pst*I. A concatemeric replicative DNA intermediate equivalent to eight mature genome lengths was identified. The concatemer was shown to be the precursor for the synthesis of mature bacteriophage DNA which is subsequently packaged by a headful mechanism.**

Information on the genetics of *Vibrio cholerae*, a highly pathogenic gram-negative bacterium and the causative agent of the disease cholera, is limited, primarily owing to the lack of demonstrable genetic exchange systems. Transformation has not been demonstrated in this organism, and the transfection efficiency is low (1). Conjugation is mediated by a factor P (4) which, unlike the F factor of *Escherichia coli*, cannot induce Hfr donors (22). A transducing bacteriophage has only recently been described from *V. cholerae* biotype *eltor*, a biotype of the classical vibrios (20). Although four serologically (18) and morphologically (5) distinct groups of phages which can infect *V. cholerae* cells have been identified, these phages have not been used so far in the analysis of the genetics of this organism. Of these phages, only phage  $\phi$ 149 belonging to group IV has been examined in some detail (23, 26) because of its taxonomic importance. This phage lyses all strains of classical vibrios but none of the *eltor* biotype (19). Phages belonging to other serological groups have not been examined yet.

While examining the process of infection by cholera phage  $\phi$ 138, a phage belonging to group II, it was observed that  $\phi$ 138 infection can induce a defective phage carried by the hypertoxinogenic strain 569B of classical vibrios (R. Chowdhury and J. Das, unpublished observation), and unlike  $\phi$ 149 (23),  $\phi$ 138 infection is not repressed by phosphate ions in the growth medium.

It has been reported that while UV-induced damage in phage  $\phi$ 149 DNA cannot be repaired by the host-cell reactivation (HCR) mechanism, using the same host system, UV-irradiated phage  $\phi$ 138 can be repaired by this mechanism (21). Host-cell reactivation is the repair of damage in phage DNA brought about by the removal of pyrimidine dimers by the host-cell excision repair mechanism and presumably does not involve any phage gene functions (3, 12). The sensitivity of UV-irradiated phages, therefore, depends on the genotype and phenotype of the host used for assay, and it is possible to produce an HCR<sup>-</sup> phenotype of HCR<sup>+</sup> cells (10, 13). Phage  $\phi$ 149 DNA has single-strand interruptions (26) along its length similar to those of coliphage T5 (27). Parenthetically, UV-irradiated phage T5 also cannot be host-cell reactivated (7). Whether the nicks in the phage DNA have any role in the host-cell reactivation phenomenon is not understood.

It is in this context that an examination of  $\phi$ 138 DNA and its intracellular replication was considered intriguing. Besides the fact that  $\phi$ 138 can infect both the classical vibrio and its biotype *eltor* and that the infection is lytic, nothing is known about the general features of its intracellular replication and about its genome. The present report shows that  $\phi$ 138 contains a linear double-stranded DNA of molecular weight  $30 \times 10^6$  or 45 kilobase pairs (kbp) and that the DNA is circularly permuted. A concatemeric replicative DNA intermediate during intracellular replication of this phage was identified.

## MATERIALS AND METHODS

**Bacteria and bacteriophage.** *V. cholerae* Ogawa 154, the universal host for propagation of cholera phages (9), was used for phage propagation. Under conditions when the spontaneous induction of defective phage was insignificant, cells of either strain 154 or the hypertoxinogenic strain Inaba 569B were used as the host for phage infection studies. Induction of defective phages carried by strain 569B occurred mostly after repeated passage of  $\phi$ 138 in nutrient broth. Infection of cells by  $\phi$ 138 was carried out in Tris-Casamino Acids (Difco Laboratories; Detroit, Mich.)-glucose medium. Bacteriophage  $\phi$ 138 belonging to group II (19) was used in this study. The efficiency of plating was not significantly different in *V. cholerae* 154 and *V. cholerae* 569B cells. Cultures were maintained as described previously (16, 24). Cell and phage growth were assayed as CFU and PFU, respectively, in nutrient agar plates.

**Media and buffers.** The nutrient broth and nutrient agar media used in these studies were as described previously (9). The Tris-Casamino Acids-glucose medium contained 50 mM Tris hydrochloride (pH 7.5), 0.5% NaCl, 5 mM MgSO<sub>4</sub>, 0.4% KH<sub>2</sub>PO<sub>4</sub>, 0.1% NH<sub>4</sub>Cl, 0.0004% FeCl<sub>3</sub> · 6H<sub>2</sub>O, 0.2% glucose, and 0.2% Casamino Acids in distilled water. Tris magnesium buffer used contained 50 mM Tris hydrochloride (pH 7.5) and 5 mM MgCl<sub>2</sub>. Label-terminating buffer contained 50 mM Tris hydrochloride (pH 7.5), 5 mM MgCl<sub>2</sub>, 2 mM NaN<sub>3</sub>, and 3 mg of thymidine per ml.

**Preparation and purification of phage.** High-titered phage stock was prepared by infecting *V. cholerae* 154 cells with  $\phi$ 138 following the method described for purification of  $\phi$ 149 (23).

For the preparation of <sup>32</sup>P-labeled phage infection was

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carried out in phosphate-depleted nutrient broth (25). [ $^3\text{H}$ ]thymidine-labeled phage was prepared by infecting cells grown in Tris-Casamino Acids-glucose medium and subsequently purified as described before (23).

**Isolation of  $\phi$ 138 DNA.** Purified phage was dialyzed against 10 mM Tris hydrochloride–1 mM EDTA (pH 8.0) buffer at 4°C for 4 h. The DNA was extracted from the phage by phenol saturated with the same buffer, and the aqueous phase was extensively dialyzed as described previously (26).

**Preparation of infected-cell lysate.** At different times during infection, samples of infected culture were withdrawn and, whenever required, labeled with 10 to 25  $\mu\text{Ci}$  of [ $^3\text{H}$ ]thymidine (specific activity, 18.8 Ci/mmol; Bhaba Atomic Research Centre, Trombay, India) per ml for the desired length of time. Labeling was terminated by adding an equal volume of ice-cold label-terminating buffer. The cells were sedimented (10,000  $\times g$ , 10 min, 4°C) and lysed by being resuspended in 1/20th the culture volume of 0.01 M Tris (pH 8.0)–0.001 M EDTA buffer containing 2% Sarkosyl NL97. The lysate was incubated at 37°C for 15 min before sedimentation analysis.

**Sucrose gradient centrifugation.** Labeled phages or infected-cell lysates were analyzed by velocity sedimentation in either neutral or alkaline 5 to 20% (wt/vol) sucrose gradients, using a Sorvall AH650 rotor at 30,000 rpm for 150 min at 15°C as described previously (26). Fractions were collected, precipitated with trichloroacetic acid, filtered, and washed first with 5% trichloroacetic acid and then with distilled water. The dried filter papers were counted in a LKB 1217/1218 Rackbeta liquid scintillation counter in a toluene-based liquid scintillation fluid containing 4 g of PPO (2,5-diphenyloxazole) and 100 mg of POPOP [1,4-bis(5-phenyloxazole)benzene] per liter. The molecular weights of phage DNA and replicative intermediates were estimated from the distances sedimented, using [ $^{14}\text{C}$ ]thymidine-labeled T4 DNA or labeled  $\phi$ 138 DNA as markers as described previously (26).

**Nuclease digestion.** The restriction endonucleases *Bgl*II, *Pst*I, and *Hpa*I and the single-strand-specific nuclease S1 (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and *Hind*III, *Eco*RI, *Xba*I, and the nuclease *Bal*31 (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) were used in these studies. Digestion of purified DNA with restriction enzymes was carried out according to the instructions of the manufacturers. All digests were heated at 65°C for 5 min and quenched in ice to melt small hydrogen-bonded overlaps before loading on gels. The digestion buffer for the enzyme BAL 31 contained 20 mM Tris, 12 mM  $\text{MgCl}_2$ , 12 mM  $\text{CaCl}_2$ , 300 mM NaCl, and 1 mM EDTA (pH 8.1). The digestion was carried out at 30°C with 0.5 U of BAL 31 per 5  $\mu\text{g}$  of DNA. Samples were removed at different times during incubation, and the reaction was stopped by adding 0.02 M (final volume) EGTA [ethylene glycol-bis( $\beta$ -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid] and chilling. The DNA was phenol extracted, ethanol precipitated, and used for digestion with restriction enzymes.

**Gel electrophoresis.** Agarose (0.8%) horizontal slab gels (30 by 13.5 by 0.5 cm) were formed in buffer containing 0.04 M Tris acetate (pH 8.0) and 2 mM EDTA. Electrophoresis was carried out at 2 V/cm of gel for 15 h at room temperature (approximately 24°C). Reaction mixtures to be electrophoresed were mixed with 1/10 volume of a solution composed of 0.4% bromophenol blue, 0.05 M sodium EDTA, and 30% Ficoll. To visualize the DNA fragments, the gels were soaked in electrophoresis buffer containing ethidium bromide (1  $\mu\text{g}/\text{ml}$ ) and photographed with a 300-nm-wavelength

transilluminator (model 3-4400; Fotodyne Inc.) and a red filter.

In some experiments, restriction enzyme digests were analyzed in gels containing 0.4 to 0.5% low-melting-temperature agarose (Bethesda Research Laboratories, Inc.) in Tris acetate buffer at 2 V/cm for 12 to 16 h at 4°C. DNA fragments from low-melting-temperature agarose gels were recovered by the method of Weislander (28).

**Electron microscopy.** Phage preparations were negatively stained with either 1% phosphotungstic acid (pH 7.0) or 2% uranyl acetate (pH 5.4). DNA was prepared for electron microscopy by the formamide technique described by Davis et al. (11). Electron micrographs were taken in a JEOL 100CX transmission electron microscope at an operating voltage of 60 kV.

## RESULTS

**Early events after infection.** Cholera phage  $\phi$ 138 consists of a polyhedral head (61 by 65 nm) and a long rigid tail (801 nm) with a pronglike structure at the end (Fig. 1a and b). The contractile tail is attached to the head by a narrow neck (arrowhead, Fig. 1b).

Infection by  $\phi$ 138 is lytic with a latent period of about 25 min, and the burst size is about 30. When [ $^3\text{H}$ ]thymidine-labeled cells were infected with  $\phi$ 138 (multiplicity of infection, 10) and acid-precipitable radioactivity was assayed at different times during infection, more than 70% of the host DNA was degraded within 2 min of infection, and phage-specific DNA synthesis started at about 20 min after infection. About 50% of the acid-precipitable radioactivity was recovered as DNase-resistant counts in the infected-cell lysate after 65 min of infection (Table 1). From the results of a typical experiment, the number of labeled thymidine molecules per cell immediately before infection was estimated to be  $1.17 \times 10^3$  (1,300 cpm/4  $\times 10^7$  CFU), and assuming the total number of thymidine molecules per cell genome to be  $3.1 \times 10^6$  (8), the ratio of cold to hot thymidine per cell was 2,650:1. Upon infection by  $\phi$ 138,  $2 \times 10^9$  infectious centers were recovered per 100  $\mu\text{l}$  of cell lysate at 75 min after infection, and the DNase-resistant radioactivity in the same sample was 644 cpm, corresponding to about 12 radioactive thymidine molecules per phage genome. Assuming the G+C content of  $\phi$ 138 DNA to be 42% (6), the ratio of cold to hot thymidine in phage DNA was 1,300:1. Thus, the phage primarily utilizes the host DNA degradation products for its own DNA synthesis.

**$\phi$ 138 genome.** DNA isolated from purified  $\phi$ 138 was examined either by velocity sedimentation through 5 to 20% (wt/vol) neutral and alkaline sucrose gradients or under the electron microscope. All the DNA molecules observed under the electron microscope were linear and double stranded (Fig. 1c). The average molecular weight of  $\phi$ 138 DNA estimated from velocity sedimentation analysis in neutral sucrose gradients was  $30 \times 10^6$  or 45 kbp (Fig. 2a). This value is in agreement with the measurements of several molecules from electron micrographs.

When  $\phi$ 138 DNA was sedimented in an alkaline sucrose gradient, two peaks were resolved (Fig. 2b). The single-stranded DNA molecular weights corresponding to material sedimenting in these two peaks were  $14.8 \times 10^6$  and  $3.9 \times 10^6$ . This result suggests that while one of the strands of  $\phi$ 138 DNA is intact, the other strand might have either apurinic sites or single-strand interruptions along its length. To investigate this possibility, the  $\phi$ 138 DNA was digested with single-strand-specific nuclease S1. However, no S1-sensitive site was detected (data not shown). As a control, cholera-

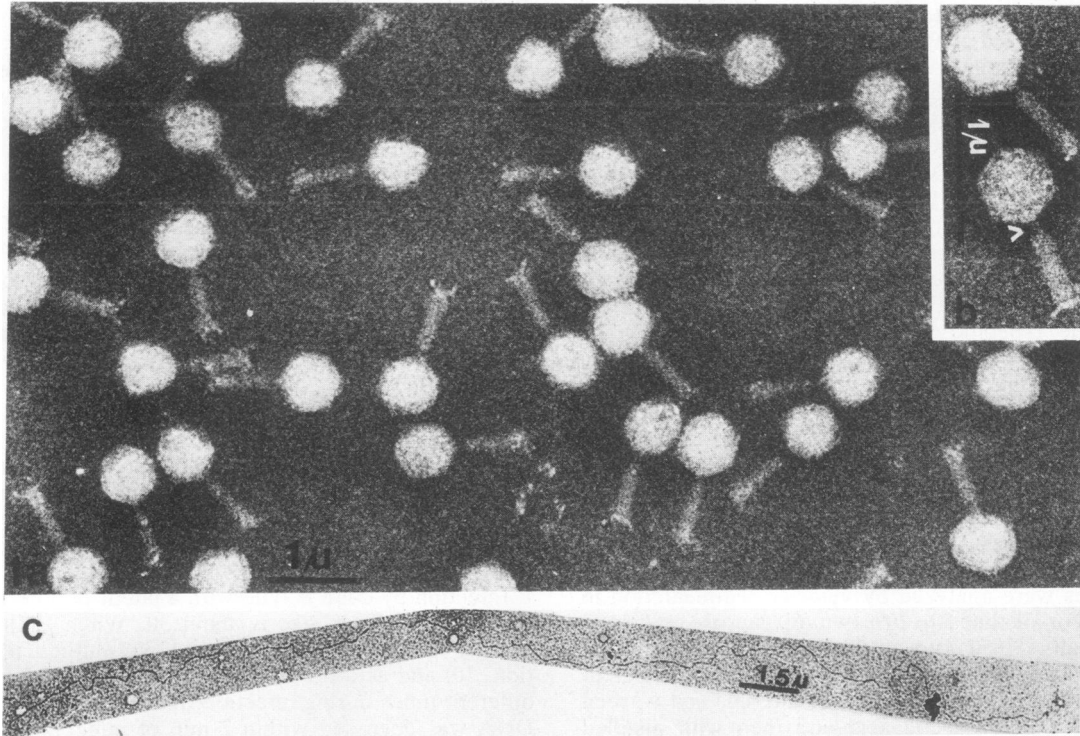


FIG. 1. Electron micrograph of  $\phi$ 138 and its DNA. (a and b)  $\phi$ 138 virions negatively stained with 1% phosphotungstic acid; arrowhead shows narrow neck. (c)  $\phi$ 138 DNA.

phage  $\phi$ 149 DNA, which has been reported to have single-strand interruptions along its length (26), was digested with S1 nuclease, and well-defined fragments were observed, in agreement with the published report (26). Thus, one of the strands of  $\phi$ 138 DNA might have three alkali-labile sites.

**Restriction enzyme analysis of  $\phi$ 138 DNA.**  $\phi$ 138 DNA was incubated with various restriction endonucleases, and the digestion products were analyzed by slab gel electrophoresis (Fig. 3). The sizes of fragments produced are listed in Table

2. The inaccuracy in estimating the sizes of large fragments was eliminated by digesting the phage DNA with two enzymes in succession. The sum of the molecular weights of the fragments obtained after digestion with different restriction enzymes varied between 44 and 46 kbp, which is in agreement with the size obtained from sedimentation analysis or electron microscopy. Densitometric analysis of the gel patterns was used to identify doublets and fragments not present in equimolar amounts. The sum of the molecular

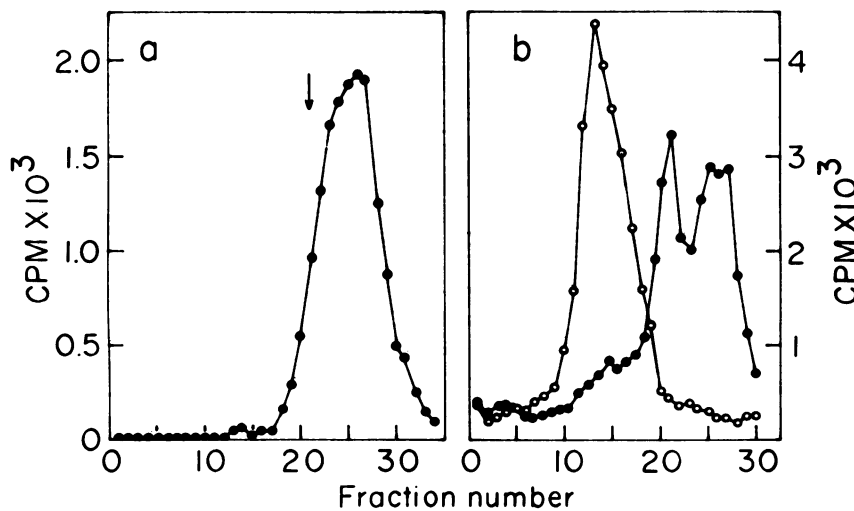


FIG. 2. Velocity sedimentation of [<sup>3</sup>H]thymidine-labeled  $\phi$ 138 DNA. The DNA was sedimented through neutral (a) and alkaline (b) 4.7-ml 5 to 20% sucrose gradients as described in Materials and Methods. Symbols: ●,  $\phi$ 138 DNA; ○, <sup>14</sup>C-labeled T4 DNA used as a marker. The arrow indicates the position of labeled marker T4 DNA. Sedimentation is from right to left.

TABLE 1. Utilization of host DNA degradation products in phage DNA synthesis<sup>a</sup>

Time after infection (min)	% Radioactivity remaining <sup>b</sup>
0	100
2	26
5	33
20	50
30	65
45	55
60	56
75	50

<sup>a</sup> Cells were labeled for 1 h in the mid-logarithmic phase of growth and starved in Tris magnesium buffer (pH 7.6) for 30 min to exhaust the cellular nucleotide pool. Cells were then infected with  $\phi$ 138 at a multiplicity of infection of 10. Five minutes were allowed for adsorption, and at different times samples were removed and assayed for acid-precipitable radioactivity. At 75 min after infection, there was 49% DNase-resistant radioactivity (incorporation of host DNA degradation product into phage DNA was assayed by measuring DNase-resistant acid-precipitable counts in cell lysate).

<sup>b</sup> 1,300 cpm/100  $\mu$ l was taken as 100%.

weights of the *Bgl*II fragments was about 49 kbp. However, the intensity of fragment D of the *Bgl*II digest was six to seven times less than those of the other fragments. Even in the limit digest of some of the enzymes, certain fragments (such as fragment C of *Pst*I, fragment C of *Hpa*I, fragment A of *Hind*III) were faint (Fig. 3). Although *Mbo*I does not have any site in the DNA, digestion with *Dpn*I and *Sau*3A produced more than 20 fragments. The enzymes *Mbo*I, *Dpn*I, and *Sau*3A are isoschizomers recognizing the sequence GATC. *Mbo*I cleaves unmethylated GATC sequences, *Dpn*I cuts only methylated GATC sequences, and *Sau*3A cleaves GATC sequences regardless of the state of methylation. Thus, the GATC sequences present in  $\phi$ 138 DNA have their adenine residues methylated. This is consistent with the fact that *V. cholerae* 569B cells are *dam*<sup>+</sup> (2).

**Circular permutation in  $\phi$ 138 DNA.** While constructing a physical map of  $\phi$ 138 DNA, terminal labeling with [ $\gamma$ -<sup>32</sup>P]ATP or digestion with the enzyme BAL 31 was used to identify the terminal sequences in the DNA. When  $\phi$ 138 DNA treated with BAL 31 for different lengths of time was cleaved with *Bgl*II within 2 min of BAL 31 digestion, fragment D of the *Bgl*II digest was no longer detectable. However, no other fragments disappeared completely up to 60 min of BAL 31 treatment, although the relative intensity of fragment C decreased first, followed by a decrease in the relative intensity of fragment B. The intensity of fragment A remained unaltered (Fig. 4). When BAL 31-digested DNA was cleaved with *Pst*I, the relative intensities of all the fragments were reduced, and none of the fragments disappeared completely up to 60 min of BAL 31 treatment (data not shown). The intensities of fragments D1 and E (Fig. 3) were reduced more relative to the other fragments. This result suggested that the DNA population was not homogeneous with respect to their termini, in which case discrete fragments would have disappeared upon restriction enzyme cleavage of BAL 31-digested DNA. In other words,  $\phi$ 138 is circularly permuted. The preferential loss in the intensities of only some of the fragments of *Bgl*II- or *Pst*I-digested  $\phi$ 138 DNA after BAL 31 treatment, rather than all of them simultaneously (Fig. 4), suggested that the permutation in  $\phi$ 138 DNA was restricted, similar to that reported for  $\phi$ 42 (17). If the DNA was randomly permuted, that is, all sequences have equal probability to be at the end, the

relative intensities of fragments produced by restriction enzyme cleavage of BAL 31-treated DNA should have remained unaltered. The restricted permutation in  $\phi$ 138 DNA might explain the presence of faint bands in complete digests of some of the restriction enzymes (Fig. 3).

To ensure the assay conditions used in the present study, experiments were repeated with adenovirus type 2 DNA, which has been shown to be a nonpermuted molecule (15). The results (Fig. 5) are in agreement with the published work. The fragments designated f, g, and k disappeared after only 15 min of exonuclease treatment at 30°C followed by digestion with the enzyme *Hind*III.

**Physical map of  $\phi$ 138 DNA.** A physical map of  $\phi$ 138 DNA was constructed by using the enzymes *Bgl*II, *Hind*III, and *Pst*I (Fig. 6). The linkages between the different fragments were determined either from the analysis of partial digestion products or from the digestion of isolated restriction fragments produced by one enzyme with a second enzyme. The double-digest pattern of  $\phi$ 138 DNA with *Bgl*II and *Pst*I (Fig. 3) showed that all the *Bgl*II fragments had *Pst*I sites and that *Pst*I fragments, A, B, and D1 were cleaved, producing six new fragments. The *Bgl*II fragments of  $\phi$ 138 DNA were eluted from low-melting-temperature agarose gels and digested with *Pst*I, and the digestion products were analyzed in 0.8% agarose gels (Fig. 7). The double-digest fragments

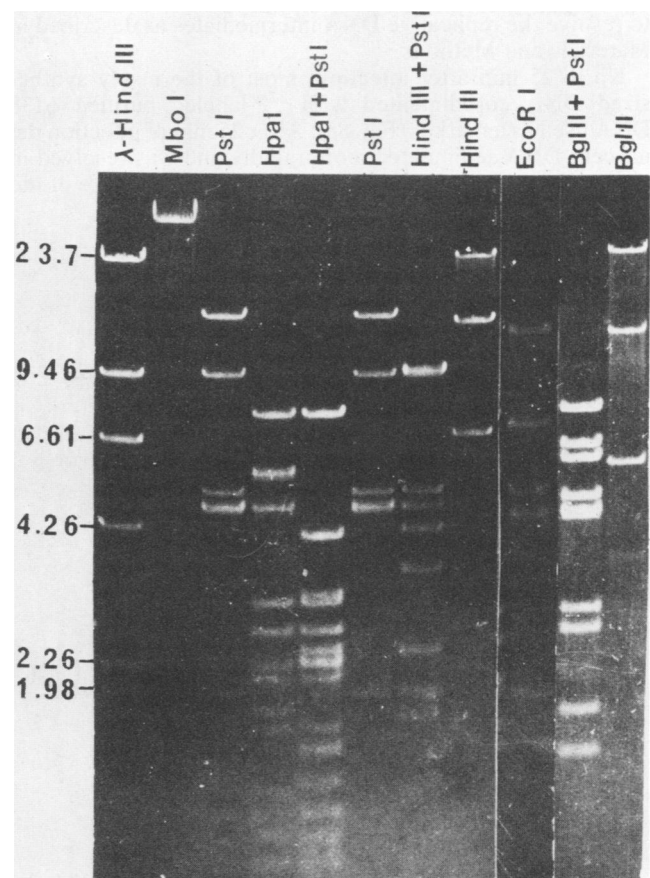


FIG. 3. Restriction enzyme digestion of  $\phi$ 138 DNA. About 1  $\mu$ g of DNA after restriction enzyme digestion as described in Materials and Methods was loaded in each lane and electrophoresed in a 0.8% horizontal slab gel. The numbers represent the molecular weight of the marker DNA fragments in kilobase pairs.

produced from each *Bgl*II fragment are summarized in Table 3. The double-digest fragments d, e1, e2, and h correspond to the C, D2, E, and F fragments, respectively, of *Pst*I. The A fragment of the *Bgl*II digest contains the *Pst*I fragments C, D2, and F, and the B fragment of *Bgl*II includes *Pst*I fragment E. The sum of the fragment sizes of a and c, b and g, and i and f of the double digest correspond to the sizes of *Pst*I fragments A, B, and D1. This indicates the linkage A-(C, D2, F)-B, A-E-D1, B-D1 between the *Pst*I fragments. The linkage between C, D2, and F was determined from the analysis of partial digestion products of *Pst*I cleavage and was found to be A-F-D2-C-B. The coupled map of *Bgl*II and *Pst*I was constructed by examining the overlaps between segments produced by the individual enzymes and their double-digest products (Fig. 3). Similarly, a *Hind*III cleavage site map was constructed by digesting  $\phi$ 138 DNA with *Hind*III and *Pst*I either singly or in succession (Fig. 6). While the *Pst*I fragments B, D1, E, and F remained uncut in the double digest, all the *Hind*III fragments had *Pst*I sites. Because of the circular permutedness of the DNA, a circular map of the linear DNA molecule is presented (Fig. 6).

**Replicative intermediates.** To further characterize intracellular events after  $\phi$ 138 infection, we examined the intracellular replication of the phage. Cells in the log phase of growth were infected with  $\phi$ 138 (multiplicity of infection, 10), and at different times during infection samples were labeled for 2 min with [<sup>3</sup>H]thymidine, and the cell lysates were analyzed on neutral 5 to 20% (wt/vol) sucrose gradients to resolve the replicative DNA intermediates as described in Materials and Methods.

Up to 25 min after infection, most of the newly synthesized DNA cosedimented with <sup>32</sup>P-labeled purified  $\phi$ 138 DNA used as a marker (Fig. 8a). After 25 min of infection the nascent DNA sedimented more rapidly and was resolved in the gradient as a sharp peak (Fig. 8b and c). The size of the

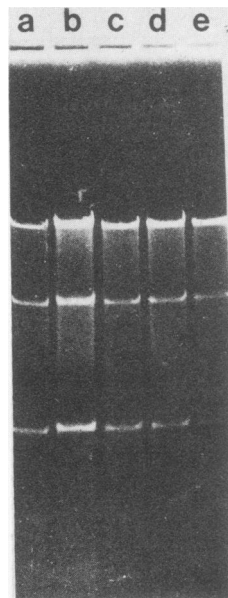


FIG. 4. Digestion of  $\phi$ 138 DNA with *Bgl*II after BAL 31 treatment.  $\phi$ 138 DNA (5  $\mu$ g) was incubated with 0.5 U of BAL 31 at 30°C. Samples were removed at 2, 10, 30, and 60 min, reactions were stopped, and the DNA was digested with *Bgl*II and analyzed by electrophoresis in a 0.8% agarose slab gel (lanes b through e, respectively). Lane a: Untreated  $\phi$ 138 DNA digested with *Bgl*II.

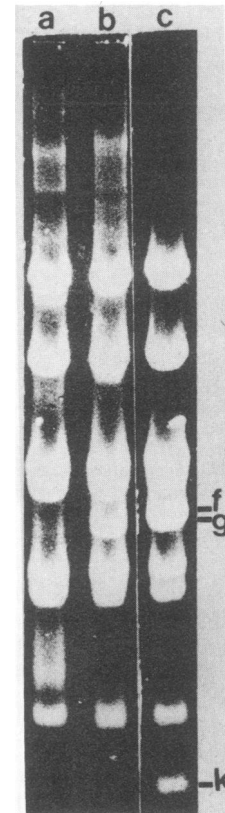


FIG. 5. Digestion of BAL 31-treated adenovirus DNA with *Hind*III. A 10- $\mu$ g sample of adenovirus type 2 DNA was digested with 1 U of BAL 31 at 30°C. Samples (10  $\mu$ l) were removed at 5 (lane b) and 15 (lane a) min and digested with *Hind*III. Lane c: Untreated DNA digested with *Hind*III. Letters on right mark fragments.

DNA molecules in the peak was estimated to be about eight genome equivalent lengths. This is consistent with the fact that the phage DNA, being circularly permuted, has terminal redundancy and can form concatemeric structures during replication.

To follow the fate of the concatemeric DNA intermediate, infected cells were pulse-labeled at 30 min after infection for 5 min with [<sup>3</sup>H]thymidine and chased throughout the lytic cycle. For chase experiments, the pulse-labeled cells were washed in ice-cold buffer containing an excess amount of cold thymidine and suspended in prewarmed fresh medium. At different times, samples were removed and analyzed on 5 to 20% (wt/vol) neutral sucrose gradients. At 35 min after infection, most of the nascent DNA was in the concatemeric form (Fig. 9a). During the chase, the molecular weight of the rapidly sedimenting intermediate decreased progressively (Fig. 9b and c), and by 30 min of chase (65 min after infection) most of the DNA sedimented as monomeric units (Fig. 9d). These results show that the concatemeric intermediate is a precursor for the synthesis of mature phage DNA.

## DISCUSSION

The results presented in this report show that cholera-phage  $\phi$ 138 contains a linear, double-stranded DNA molecule which is circularly permuted. From velocity sedimentation, electron microscopy, and restriction analysis the molecular weight of the phage DNA was estimated to be  $30 \times 10^6$  or 45 kbp. This value is much less than that reported

TABLE 2. Size of  $\phi$ 138 DNA fragments generated upon digestion with different restriction endonucleases<sup>a</sup>

Fragments	<i>Xba</i> I	<i>Hind</i> III	<i>Bgl</i> II	<i>Eco</i> RI	<i>Pst</i> I	<i>Hpa</i> I	<i>Hind</i> III + <i>Pst</i> I	<i>Bgl</i> II + <i>Pst</i> I
A	25.3	24.65	26.43	14.05	14.88	7.2		7.77
A1							9.46	
A2							9.46	
B	19.9	14.15	12.48	7.4	9.46	5.4	5.21	6.61
C		7.03	6.18	5.20	5.2	5.3 <sup>b</sup>	4.71	6.05
D			3.85 <sup>b</sup>	5.15		4.7	4.26	5.21
D1					4.77			
D2					4.77			
E				4.8	4.3	3.1	3.5	
E1								4.7
E2								4.7
F				4.7	1.98	2.75	3.16	3.32
G				2.05		2.4	1.98	2.86
H				1.7		2.25	1.82	1.98
I						2.1	1.5	1.73
J						1.93		
K						1.55		
L						1.5		
M						1.4		
N						1.33		
O						1.2		

<sup>a</sup> Fragment sizes are given in kilobase pairs. Phage  $\lambda$  *Hind*III fragments were used as a marker.

<sup>b</sup> Represents faint bands.

previously ( $1.12 \times 10^8$  daltons) from renaturation kinetic measurements (6). Considering that the circularly permuted  $\phi$ 138 DNA is packaged into the mature phage by a headful mechanism,  $1.12 \times 10^8$ -dalton DNA appears too large to be packaged in the phage head (Fig. 1). The large value reported previously might be due to formation of catenated structures during denaturation and renaturation experiments. It is interesting to note that the two other cholera phages which

have been examined so far also contain restricted circular permutation in their DNA (14, 26).

In constructing the physical map with *Bgl*II, fragment D was not considered, primarily because its intensity was only 0.14 relative to that of the other bands. In view of the fact that the size of the concatemeric intermediate estimated in

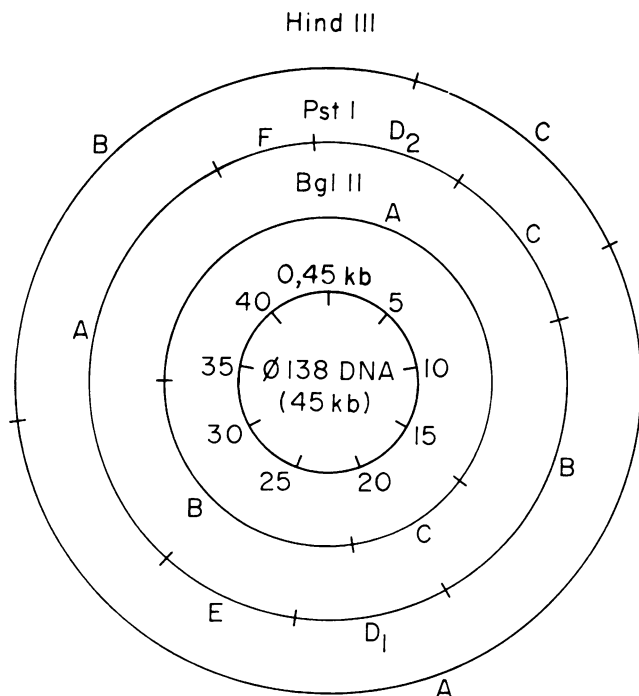


FIG. 6. *Bgl*II, *Pst*I, and *Hind*III cleavage site map of  $\phi$ 138 DNA. The inner circle represents the unit set of  $\phi$ 138 genes (approximately 45 kbp). All restriction sites have been drawn to scale.



FIG. 7. Electrophoretic separation of purified *Bgl*II fragments digested with *Pst*I. *Bgl*II fragments of  $\phi$ 138 DNA were eluted from low-melting-temperature agarose gels, and 0.5 to 1  $\mu$ g of each fragment was cleaved with *Pst*I. The products were analyzed by electrophoresis through a 0.8% agarose gel. Lanes a through c: *Bgl*II fragments A, B, and C, respectively, digested with *Pst*I.

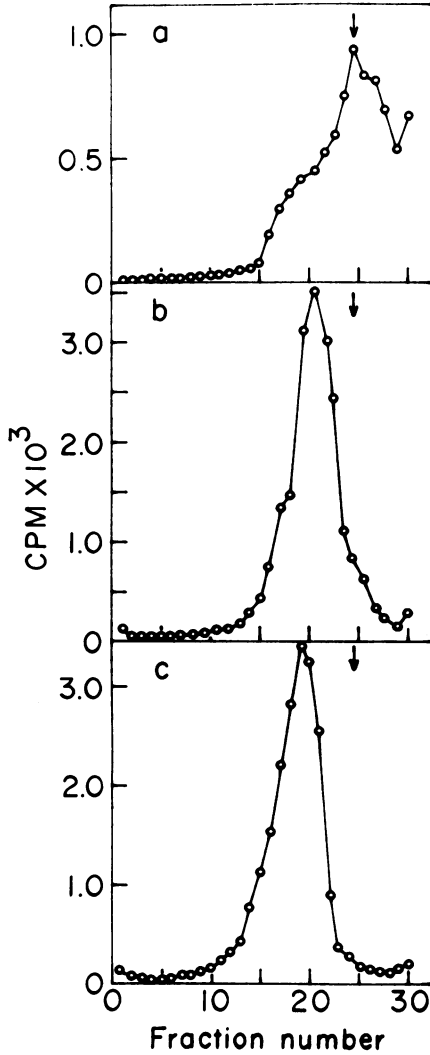


FIG. 8. Velocity sedimentation analysis of phage DNA intermediates during intracellular replication. Cells in the logarithmic phase of growth were infected with  $\phi 138$  at a multiplicity of infection of 10 as described in Materials and Methods. At 5 (a), 30 (b), and 45 (c) min after infection cells were labeled for 2 min with 25  $\mu\text{Ci}$  of [ $^3\text{H}$ ]thymidine per ml, and the cell lysates were analyzed in 5 to 20% neutral sucrose gradients. The arrows indicate  $^{32}\text{P}$ -labeled  $\phi 138$  DNA which was used as a marker. The sedimentation is from right to left.

the present study is eight phage equivalent genome lengths, it appears that fragment D of the *Bgl*II digest might be present once in every concatemer. Furthermore, this fragment disappeared within 2 min of BAL 31 digestion.

In contrast to infection by  $\phi 149$  (23),  $\phi 138$  infection is not repressible by the concentration of phosphate ions in the

TABLE 3. Digestion of *Bgl*II fragments with *Pst*I<sup>a</sup>

<i>Bgl</i> II + <i>Pst</i> I	a	b	c	d	e1	e2	f	g	h	i
<i>Bgl</i> II fragment A	x	x		x	x					x
<i>Bgl</i> II fragment B			x			x				x
<i>Bgl</i> II fragment C							x	x		

<sup>a</sup> x indicates the presence of the corresponding *Pst*I fragments in the *Bgl*II fragment.

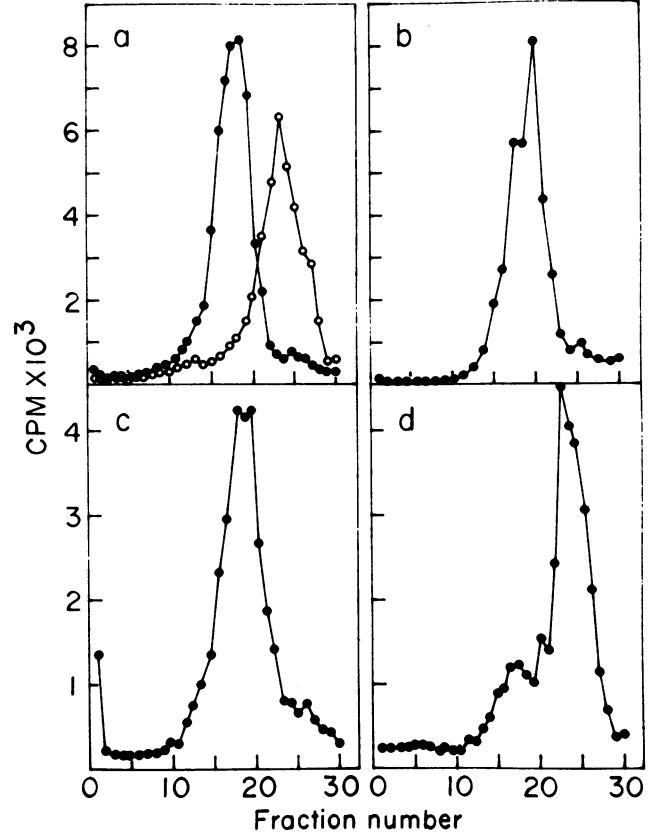


FIG. 9. Velocity sedimentation analysis of  $\phi 138$ -infected cells pulse-labeled for 5 min with 25  $\mu\text{Ci}$  of [ $^3\text{H}$ ]thymidine per ml at 30 min after infection (a) as described in the legend to Fig. 8 and chased for 10 (b), 20 (c), and 30 (d) min during infection. The cell lysates were analyzed in 5 to 20% neutral sucrose gradients. Symbols: ●, pulse-labeled DNA; ○,  $^{32}\text{P}$ -labeled  $\phi 138$  DNA used as a marker. Sedimentation is from right to left.

growth medium (data not shown). Besides, unlike  $\phi 149$ , UV-induced DNA damage in  $\phi 138$  can be host-cell reactivated by the same host system (21). Structurally, the DNAs of these two cholera phages are similar except that  $\phi 149$  DNA has single-strand interruptions along its length which are repairable by T4 DNA ligase (26). Whether the presence of nicks in the DNA has any role in the host-cell reactivation phenomenon is unknown.

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