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Vibrio cholerae Bacteriophage CP-T1: Characterization of Bacteriophage DNA and Restriction Analysis

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Temperate bacteriophage CP-T1 of *Vibrio cholerae* has a capsid that is 45 nm in diameter, a contractile tail 65 nm long and 9.5 nm wide, and a baseplate with several spikes or short tail fibers. The linear double-stranded DNA is 43.5 ± 1.4 kilobases long, and the phage genome is both terminally redundant and partially circularly permuted. The extent of terminal redundancy is ca. 4%, and circular permutation is up to ca. 44%. Circular restriction maps have been constructed for the enzymes *Hin*dIII, *Eco*RI, *Bam*HI, and *Pst*I. By restriction endonuclease and heteroduplex analyses of phage DNA, the presence and location of a site (*pac*) at which packaging of phage DNA is initiated was established.

Genetic studies of *Vibrio cholerae* have been very limited. Conjugal DNA transfer by the P sex factor (3) has been the classical procedure used in genetic analysis of the *V. cholerae* chromosome (2, 20). This has been refined by the use of transposable elements inserted into both the *V. cholerae* chromosome and the P sex factor. These transposons provide homology for the P sex factor to integrate into the chromosome producing Hfr strains which transfer the region adjacent to the transposon at a much higher frequency than conventional Hfr strains (11, 12).

More recently, identification in *V. cholerae* of two Mu-like bacteriophages has facilitated these studies. Vibriophages VcA1 and VcA2 (5) and their derivatives have provided a method for the isolation of a large number of bacterial mutants (10, 14).

Another bacteriophage, CP-T1, has been demonstrated to mediate transfer of genetic markers by a generalized mechanism of transduction (19). This property of the phage, coupled with the fact that it is temperate and can propagate on both classical and El Tor biotypes of *V. cholerae* (7, 18), makes it an important potential tool in increasing our genetic understanding of its host.

Bacteriophage CP-T1 also possesses the intriguing ability to mediate serotype conversion within *V. cholerae* (18, 19). Lysogeny of Ogawa serotype strains is accompanied by the appearance of the Inaba-specific antigenic determinant, factor C; that is, strains containing antigenic determinants A and B are converted to the Hikojima serotype, which possesses antigenic factors A, B, and C, all of which reside in the lipopolysaccharide. Whether or not this conversion plays a role in immunity to superinfection by homologous phage is unknown. The mechanism of this serotype conversion is also not understood.

Work in this laboratory is currently aimed at elucidating the mechanism of serotype conversion and gaining an insight into the molecular biology of bacteriophage CP-T1. Its temperate nature and its ability to grow on both *V. cholerae* biotypes suggest that it may be feasible to develop it as a *V. cholerae*-specific cloning vehicle.

In this paper we describe the nature of the DNA of bacteriophage CP-T1, present a restriction endonuclease cleavage map of the DNA, and discuss the mechanism by which the phage packages its DNA.

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MATERIALS AND METHODS

Bacteria and bacteriophage. *V. cholerae* El Tor strain 1621 (serotype Ogawa) was used as an indicator strain for phage CP-T1, both of which were generously provided by J. E. Ogg.

Phage preparation. Wild-type CP-T1 was prepared from 1 liter of strain 1621 grown in brain heart infusion (Difco Laboratories, Detroit, Mich.) to a density of 2×10^8 cells per ml. CP-T1 was added at a multiplicity of infection of 0.1, and the phage was collected after lysis (4 to 5 h). Residual bacteria were removed by centrifugation at 10,000 × *g* for 10 min in a GSA rotor (Ivan Sorvall, Inc., Norwalk, Conn.) at 4°C. The phages were pelleted by centrifugation at 10,000 × *g* for 4 h at 4°C in a GSA rotor (Sorvall), and the pellets were resuspended in 4 ml of the supernatant by standing overnight at 4°C. Bacteriophages were purified by centrifugation on a CsCl gradient (made from three steps consisting of 39, 45, and 51% CsCl [wt/wt] in 10 mM Tris-hydrochloride–1 mM $MgSO_4$, pH 7.5), in an SW41 Ti rotor at 30,000 × *g* for 90 min at 20°C in a Beckman L8-80 ultracentrifuge.

Bacteriophage CP-T1 DNA was obtained by two extractions of 100 μl of purified phage (usually 10^{13} PFU/ml) diluted fivefold in TE buffer (10 mM Tris-hydrochloride, 1 mM EDTA, pH 8.0) with equal volumes of TE buffer-saturated phenol, and then by two extractions with ether. DNA was dialyzed overnight against 1 liter of TE buffer.

Plasmids. Plasmids used were pPM401, pPM403, and pPM404. These are the 4.2-kilobase (kb), 1.38-kb, and 0.64-kb *Pst*I fragments of CP-T1 cloned into the *Pst*I site in the β-lactamase gene of pBR322 (A. Guidolin and P. A. Manning, submitted for publication).

Enzymes. Restriction endonucleases *Hin*dIII, *Bam*HI, *Sma*I, and *Bgl*II were purchased from Boehringer-Mannheim, Sydney, Australia. *Eco*RI was from New England Nuclear Corp., Boston, Mass., and *Pst*I was from Amersham, Buckinghamshire, England. *Xho*I and *Kpn*I were obtained from P-L Biochemicals, Inc., Milwaukee, Wis. All enzymes were used as described below.

Digestion of DNA by restriction endonucleases. Cleavage reactions of the restriction enzymes *Hin*dIII, *Bam*HI,

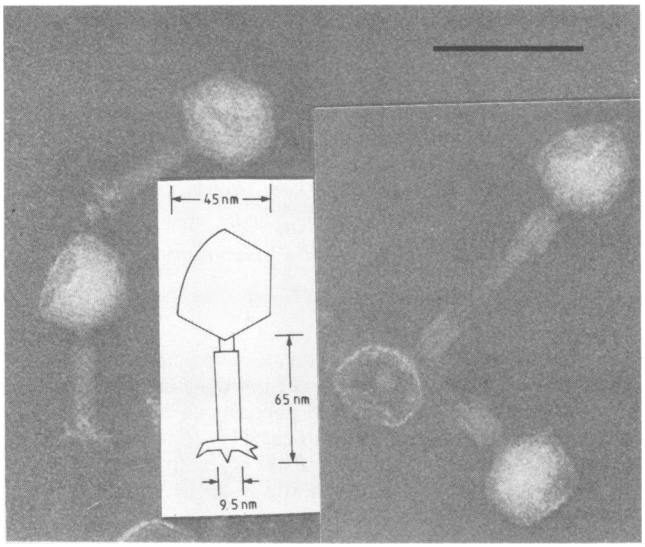

FIG. 1. Electron micrograph showing purified bacteriophage CP-T1 particles, with particles showing full and empty capsids with contractile tails. Insert: drawing showing dimensions of CP-T1 whole particles (determined relative to phage SPP1). Bar, 0.1 μm.

*Eco*RI, and *Pst*I were performed with SPK buffer (10× stock is 200 mM Tris-hydrochloride, pH 8.0, 50 mM $MgCl_2$, 5 mM dithioerythritol, 1 mM EDTA, 500 mM KCl, 50% glycerol). The remaining restriction digests were carried out as described in Davis et al. (4). Phage DNA or CP-T1 restriction fragments (0.1 to 0.5 μg) were incubated with 1 U of each restriction enzyme in a final volume of 20 μl at 37°C for 1 to 2 h. The reactions were terminated by heating at 65°C for 10 min. Before loading onto a gel, a 1/10 volume of tracker dye (15% Ficoll, 1 mg of bromophenol blue per ml) was added.

Analytical and preparative separation of restriction fragments. Electrophoresis of digested DNA was carried out at room temperature on horizontal 0.6% agarose gels (Seakem HGT; FMC Corp., Rockland, Maine), 13 cm long, 13 cm wide, and 0.7 cm thick. Gels were run at 100 V for 4 to 5 h in TBE buffer (67 mM Tris base, 22 mM boric acid, 2 mM EDTA, final pH 8.8). After electrophoresis, the gels were stained in distilled water containing 2 μg of ethidium bromide per ml. DNA bands were visualized by transillumination with UV light and photographed on either Polaroid 667 positive film or 665 negative film.

For preparative gels Sea Plaque (FMC Corp.) low-gelling-temperature agarose at a concentration of 0.6% was used for separation of restriction fragments. DNA bands were excised, and the agarose was melted at 65°C. Five volumes of 20 mM Tris-hydrochloride–1 mM EDTA (pH 8.0) buffer was added, and the agarose was extracted with phenol-water and then phenol-chloroform (both 1 g/ml). Residual phenol was removed with chloroform, and the DNA was precipitated with 2 volumes of ethanol and 1/10 volume of 3 M sodium acetate (pH 5.0).

Restriction fragment nomenclature. The restriction fragments generated by different enzymes were numbered sequentially from the largest to the smallest according to their relative mobilities on agarose gels (e.g., *Pst*I-1, *Pst*I-2, etc.).

Size determination of restriction fragments. The sizes of restriction fragments were determined by comparing their relative mobilities on the gels to those of DNA molecules of known size. The standards used were λ DNA cleaved with *Hin*dIII (21) and *Bacillus subtilis* phage SPP1 cut with *Eco*RI (22). Sizes of large fragments were calculated from the sum of the sizes of their subfragments obtained after digestion with a second enzyme.

Electron microscopy. DNA was spread in 0.5 M ammonium acetate at neutral pH following the method described in reference 13.

Preparation of heteroduplex DNA. DNA hybridization and spreading was performed according to the previously described method (8), with modifications as previously described (15, 16).

Negative staining. Phage preparations were stained with 2% phosphotungstic acid (pH 7.0). Electron micrographs were taken in a Philips EM301 transmission electron microscope.

RESULTS

Electron microscopy. Electron microscopic analysis of purified CP-T1 phage particles enabled details of the phage structure to be obtained. From Fig. 1 it can be seen that the phage consists of a polyhedral capsid connected to a contractile tail with a baseplate. The capsid is 45 nm in diameter, and the tail is 65 nm long and 9.5 nm wide. Upon contraction of the tail sheath, it could be seen that the tail is connected via a small neck to the capsid. The baseplate was not easily resolved, but it appeared to have several (four to six) spikes or short tail fibers.

The encapsulated nucleic acid is a single, linear, double-stranded DNA molecule 43.5 ± 1.4 kb (28.79 ± 0.9 × 10^6 daltons) in length (Fig. 2).

Terminal redundancy. Upon denaturation and renaturation of the linear phage DNA, double-stranded circular molecules with two single-stranded tails were formed (Fig. 3). The occurrence of such molecules implies that the DNA is both terminally redundant and circularly permuted.

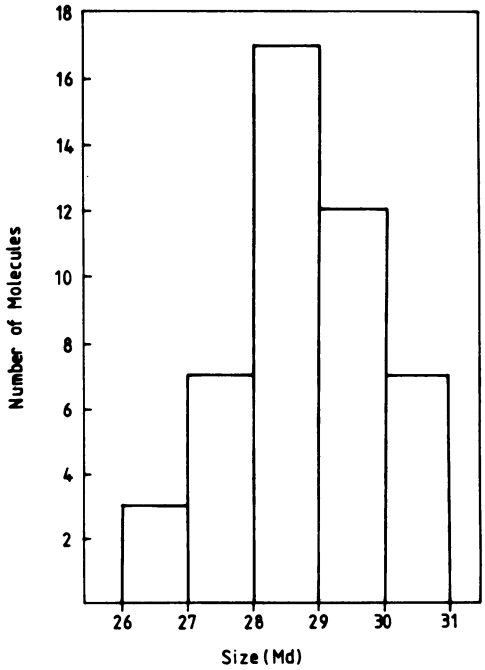

FIG. 2. Histogram of contour length of CP-T1 DNA. Plasmid ColE1 (4.2 × 10^6 daltons) was used as the double-stranded DNA standard.

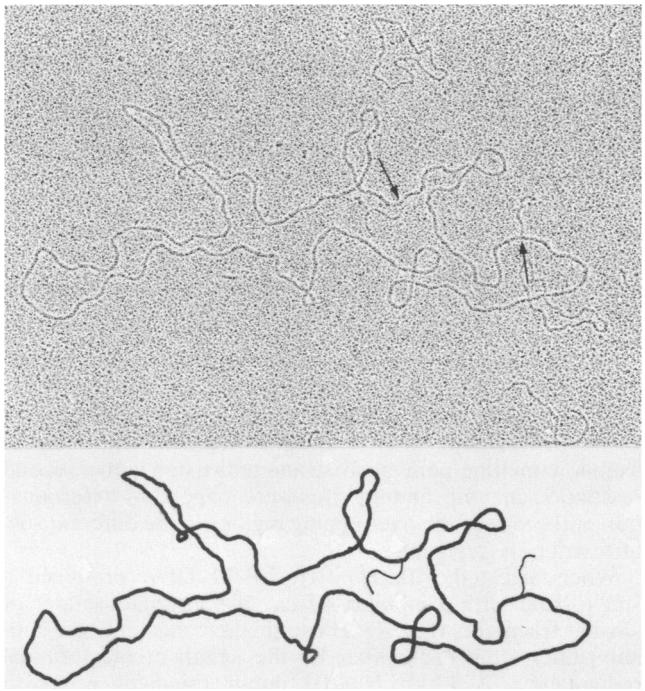

FIG. 3. Electron micrograph of circular CP-T1 DNA homoduplex, obtained after denaturation and renaturation of CP-T1 DNA. Arrows point to single-stranded tails.

The extent of the terminal redundancy can be measured and corresponds to the length of the single-stranded tails. The results from this analysis also enable the size of the circular phage genome to be measured; that is, the terminal redundancy, which is somewhat variable between molecules, can be excluded. The results of these measurements are shown in Fig. 4. The terminal redundancy comprises ca. 4% of the genome or 1.8 ± 0.3 kb (1.2 ± 0.2 × 10^6 daltons), and the size of the genome is 41.2 ± 1.2 kb (27.3 ± 0.8 × 10^6 daltons).

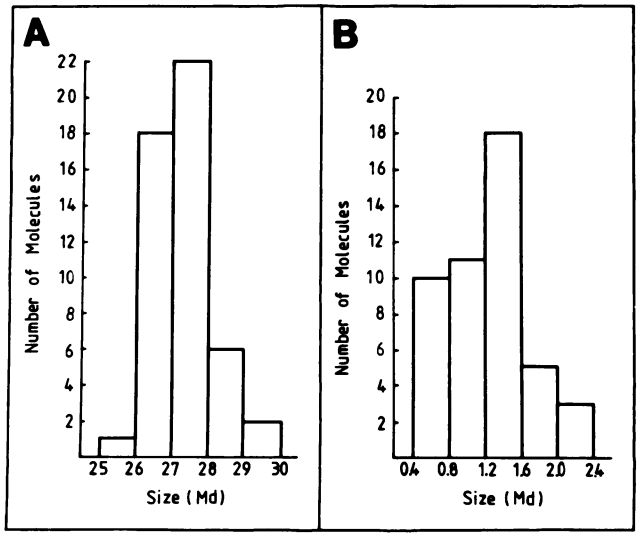

FIG. 4. Histogram obtained from the analysis of CP-T1 DNA homoduplexes. (A) Contour lengths of double-stranded circular CP-T1 homoduplexes. (B) Contour lengths of single-stranded tails formed on CP-T1 DNA homoduplexes. Plasmid ColE1 was used as reference for double-stranded DNA measurements, and φX174 DNA was used as the single-stranded DNA standard.

Circular permutation. The homoduplex analysis performed above demonstrated that the phage genome is circularly permuted. To more accurately define the degree of circular permutation, heteroduplex analysis between whole phage DNA and phage DNA fragments cloned into the plasmid vector pBR322 was performed.

The strategy was to examine the distribution of particular cloned fragments of the phage DNA along the length of different wild-type linear molecules. This was done in the following way. Three plasmids, pPM401, pPM403, and pPM404, each carrying different CP-T1 *Pst*I restriction fragments cloned into pBR322, were cut with a restriction enzyme that did not cleave the cloned DNA and cleaved the vector DNA at a single site. In each case, treatment of these plasmids with *Hin*dIII provided linear molecules which possessed asymmetrical lengths of vector DNA flanking the cloned CP-T1 DNA. Thus, upon formation of heteroduplexes, all linear phage DNA molecules could unambiguously be assigned arbitrary left and right ends. Such a heteroduplex molecule formed between linear CP-T1 DNA and pPM403 is shown in Fig. 5. The regions of homology were used to align the heteroduplexes (Fig. 6). The extent of circular permutation was then calculated as the distance between the longest and shortest arm on either the left or right end of the molecules.

Figure 6A shows the distribution of pPM401 (4.2-kb *Pst*I-5 clone) along the linear phage molecule. Twenty-four CP-T1/pPM401 heteroduplex molecules were examined. The length of DNA between the right end of the top molecule in Fig. 6A and the right end of the bottom molecule is 16.8 kb, representing 38% of the CP-T1 DNA.

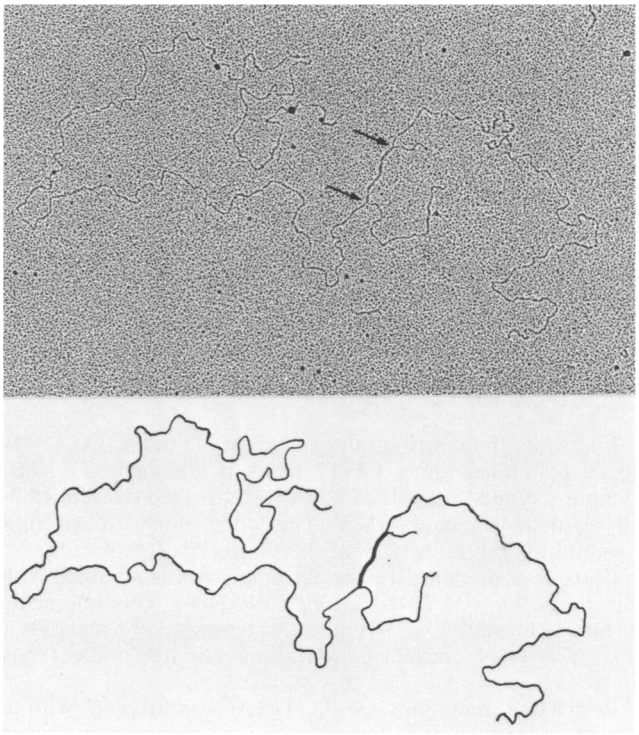

FIG. 5. Heteroduplex of linear CP-T1 DNA with the plasmid pPM403 linearized with *Hin*dIII. Arrows indicate the ends of the region of homology (thick line).

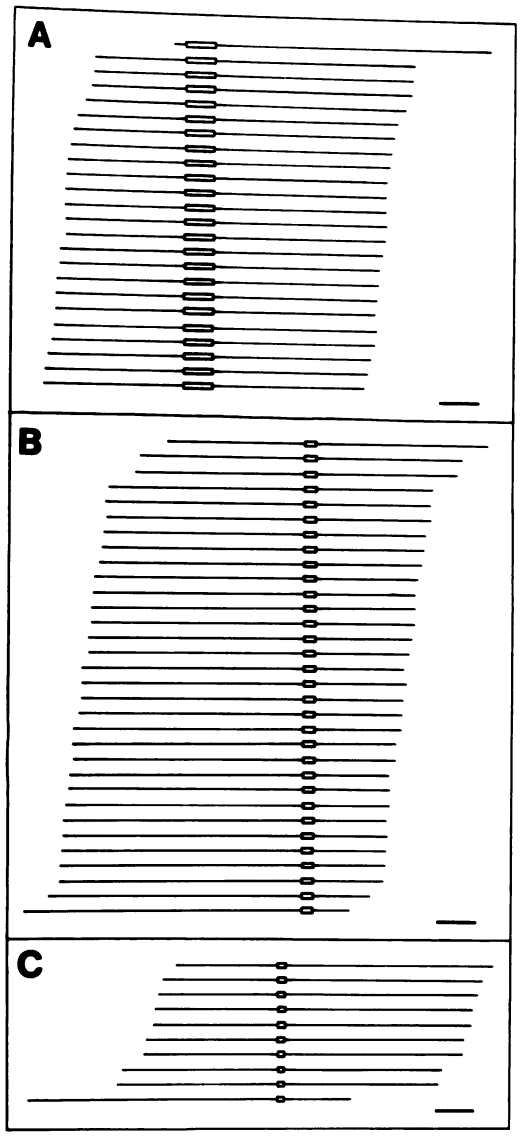

FIG. 6. Schematic representation of heteroduplexes formed between linear CP-T1 DNA and the plasmids pPM401, pPM403, and pPM404, each linearized with *Hin*dIII. (A) CP-T1/pPM401 heteroduplexes; (B) CP-T1/pPM403 heteroduplexes; (C) CP-T1/pPM404 heteroduplexes. Lengths of the single-stranded CP-T1 DNA were measured relative to ϕX174 DNA. Boxes indicate the region of homology. Bar, 5 kb.

Likewise, the distribution of 32 pPM403 molecules (1.38-kb *Pst*I-7 clone) along CP-T1 DNA is shown in Fig. 6B. Double-stranded regions of DNA were formed over an 18.3-kb segment of phage DNA. This corresponds to circular permutation covering 42% of the phage DNA molecule.

Forty-four percent circular permutation was obtained with the third 0.64-kb *Pst*I-8 clone, pPM404. This molecule formed heteroduplexes over a 19.4-kb portion of phage DNA (Fig. 6C). Thus, circular permutation is limited and extends up to 44% of the CP-T1 DNA molecule.

Restriction mapping. CP-T1 DNA was treated with a variety of restriction endonucleases, and the fragments were separated by agarose gel electrophoresis. Analysis of CP-T1 DNA treated with *Eco*RI, *Bam*HI, and *Pst*I showed the appearance of six, four, and nine restriction fragments, respectively (Fig. 7). The sizes of these fragments are summarized in Table 1.

Several anomalies in the banding pattern of CP-T1 DNA that was cleaved with restriction enzymes were apparent. Densitometer tracings (Fig. 7) revealed that fragments *Eco*RI-3, *Pst*I-1, and *Pst*I-2 were present in slightly lower molar amounts than expected. In addition, densitometer scans of *Bam*HI and *Pst*I tracks showed fragments that were present in much lower than expected molar ratios (*Bam*HI-3 and *Pst*I-4b). These 'minor' fragments are thought to represent a subset of fragments found at the ends of the mature linear DNA molecule. The significance of these fragments will be considered in the discussion.

The order of the restriction endonuclease cleavage sites for *Eco*RI, *Bam*HI, and *Pst*I within CP-T1 DNA was determined from the three possible combinations of double digests. In addition, each restriction fragment was isolated from low-melting-point agarose and redigested with a second restriction enzyme, and the sites were mapped by determination of the size of the overlapping regions in the different sets of restriction fragments.

When digested with *Hin*dIII, CP-T1 DNA produced a single band with a mobility of ca. 30 kb, plus a ladder of smaller fragments (Fig. 7). These smaller fragments presumably differ from each other by the length of the terminal redundancy. A 13-kb *Hin*dIII minor fragment was also observed. Double digests of *Hin*dIII with *Eco*RI, *Bam*HI, and *Pst*I demonstrated the presence of a single *Hin*dIII recognition sequence in the CP-T1 genome. Fragments *Eco*RI-1, *Bam*HI-1, and *Pst*I-3 were cleaved by *Hin*dIII once, allowing this site to be defined.

*Hin*dIII cleaved CP-T1 DNA once only and was therefore used as a reference or zero point in the restriction map of the phage DNA. The maps constructed with *Hin*dIII, *Eco*RI, *Bam*HI, and *Pst*I are depicted in Fig. 8. No restriction sites for the enzymes *Bgl*II, *Sma*I, *Xho*I, and *Kpn*I were detected in CP-T1 DNA.

DISCUSSION

Electron microscopic analysis of CP-T1 DNA demonstrated that the DNA was terminally redundant and that there was limited circular permutation of the genome, representing 4% and 44% of the genome, respectively. This suggests that concatemers of up to about 11 genome lengths are generated during phage DNA replication. As a consequence of the circular permutation, the linear genome, and therefore its restriction map, is rendered formally circular.

Cleavage of CP-T1 DNA by the restriction endonucleases *Hin*dIII, *Eco*RI, *Bam*HI, and *Pst*I into one, six, three, and eight major fragments, respectively, implies the same number of recognition sequences for each enzyme, due to the formal circularity of the genome. The cleavage sites for these restriction enzymes were mapped relative to each other. The molecular weight of 42.67 ± 1.3 kilodaltons for the CP-T1 size of 42.67 ± 1.3 kb for the CP-T1 genome obtained from the summation of the sizes of the restriction fragments corresponds well with the size of 41.73 ± 1.2 kb conferred by electron microscopy. A figure of 42 kb was used to draw the restriction map.

The appearance of minor fragments upon digestion of CP-T1 DNA with restriction enzymes is indicative that phage DNA contains limited circular permutation and has been reported for several bacteriophages (9, 22, 23). These fragments can be explained if packaging of DNA into the phage capsid commences from a unique initial packaging site, *pac*, on each DNA concatemer. The sizes of the *Bam*HI, *Pst*I,

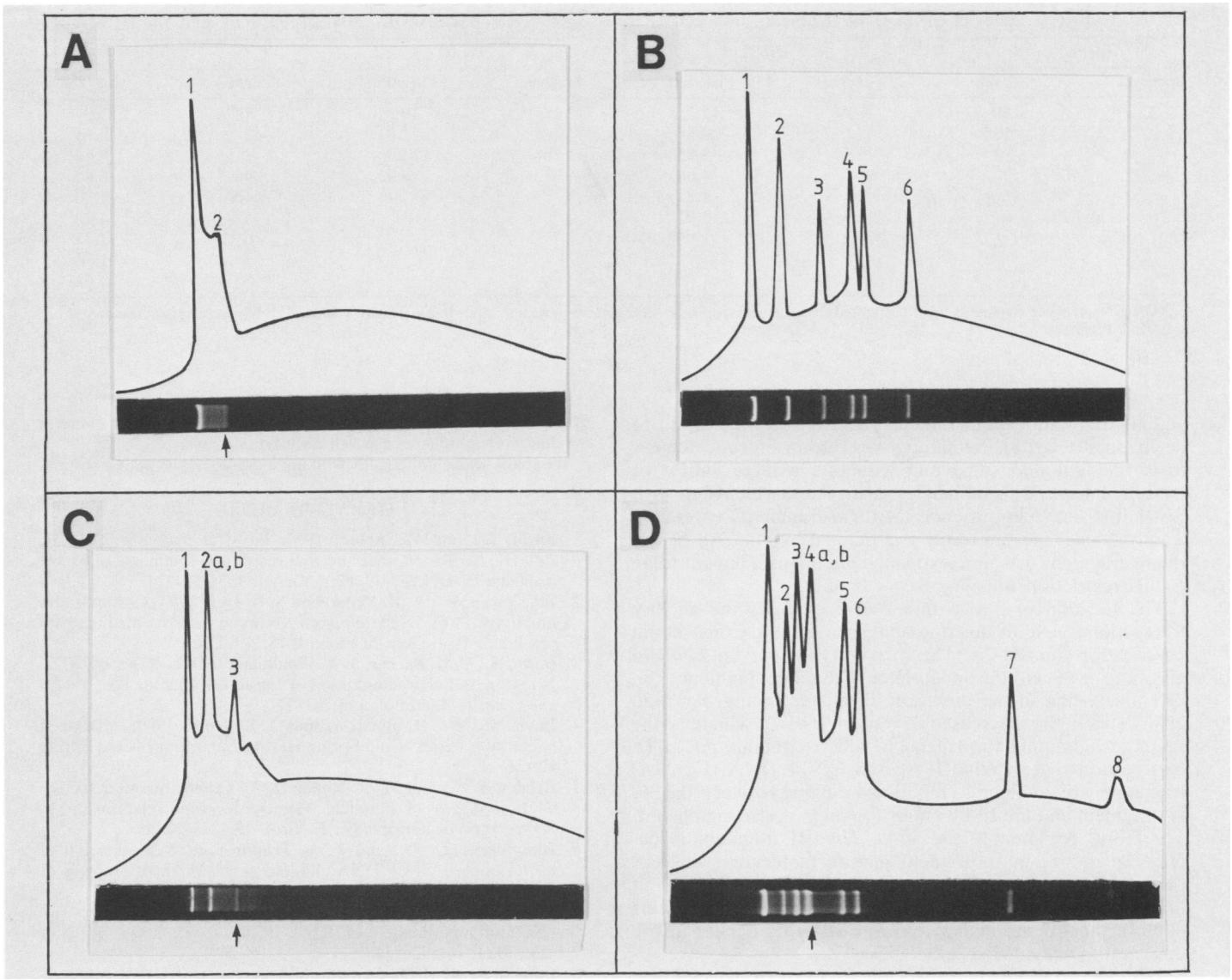

FIG. 7. CP-T1 restriction fragments that were obtained after digestion with (A) *Hin*dIII, (B) *Eco*RI, (C) *Bam*HI, and (D) *Pst*I and analyzed by agarose gel electrophoresis, followed by staining with ethidium bromide to visualize the DNA fragments. Densitometer tracings are aligned with a photograph of the digest. Arrows indicate positions of minor fragments.

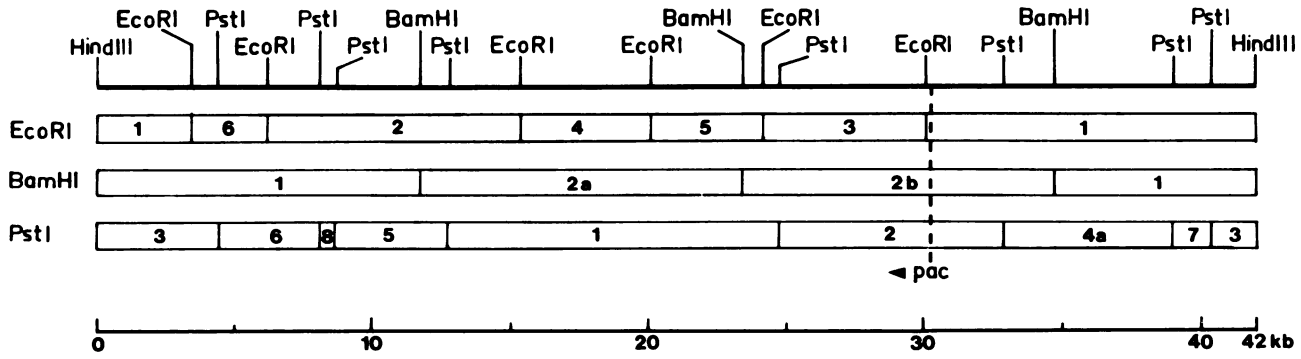

FIG. 8. Restriction map of the CP-T1 genome for the enzymes *Hin*dIII, *Eco*RI, *Bam*HI, and *Pst*I. The map shown is a linear representation, with the single *Hin*dIII site as the reference point.

TABLE 1. Sizes of CP-T1 DNA fragments produced by the restriction endonucleases *Hin*dIII, *Eco*RI, *Pst*I, and *Bam*HI

*Hin*dIII		*Eco*RI		*Bam*HI		*Pst*I	
Fragment	Size (kb)	Fragment	Size (kb)	Fragment	Size (kb)	Fragment	Size (kb)
1	30	1[a]	15.7	1[a]	19.8	1[a]	13.0
2	13[b]	2[a]	9.3	2a[a]	12.0	2	7.65
		3	5.93	2b[a]	12.0	3	6.67
		4	4.66	3[b]	6.95	4a	6.00
		5	4.21			4b[b]	5.80
		6	3.01			5	4.20
						6	3.77
						7	1.38
						8	0.64

[a] The sizes of these fragments were calculated from the sizes of their subfragments obtained after digestion with a second restriction endonuclease.
[b] Minor fragment.

and *Hin*dIII minor fragments have allowed *pac* for CP-T1 to be mapped (Fig. 8). Sequential packaging of concatemers from the first *pac* site would generate a large subset of mature, linear DNA molecules, which upon cleavage with restriction enzymes produce identical fragments extending from the cleavage site to the end defined by *pac*. An *Eco*RI minor fragment was not seen since *pac* maps adjacent to an *Eco*RI restriction site (Fig. 8).

The location of *pac* within *Pst*I-2 explains the slightly lower molar yield of this fragment, and similarly this should be true for *Bam*HI-2b. The proximity of *pac* to *Eco*RI-3 gives this fragment the appearance of a minor fragment (Fig. 7B). Packaging in the direction illustrated in Fig. 8 would also explain the decreased levels of *Pst*I-1, which is the fragment adjoining the fragment which contains *pac*. The observation that in *Hin*dIII-treated CP-T1 DNA (Fig. 7A) fragments are confined to mobilities ranging between the 30-kb fragment and the 13-kb minor fragment is also significant. Therefore, presumably the 30-kb *Hin*dIII fragment is decreasing in size in subsequent mature molecules, whereas the 13-kb minor fragment is increasing in size, by unit increments corresponding to the terminal redundancy. This adds further to the evidence that packaging occurs in the direction indicated (Fig. 8).

With the accurate analysis of the first mature phage DNA molecule determined, one could calculate the expected sizes of fragments extending from the end opposite *pac* to the restriction cleavage sites. Such fragments should be present in equimolar amounts to the *pac*-generated minor fragments. These expected fragments were not detected at the calculated positions in the *Eco*RI and *Pst*I digests, but a diffuse band which peaks at the predicted size of 5.9 kb in the *Bam*HI digest, rather than a more intense unique band, has been observed (Fig. 7C). Similar observations have been made for phages P22, P1, and SPP1 (1, 6, 9) and are caused by imprecision in the endpoint cutting during headful packaging. This would also explain the heterogeneity in the observed length of terminal redundancy seen in electron micrographs of CP-T1 homoduplex molecules (data not shown).

These studies provided a basis with which to modify the CP-T1 genome to construct a recombinant DNA vector. The presence of a single *Hin*dIII cleavage site within the phage genome may provide a potential cloning site.

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LITERATURE CITED

1. **Bächi, B., and W. Arber.** 1977. Physical mapping of *Bgl*II, *Bam*HI, *Eco*RI, *Hin*dIII and *Pst*I restriction fragments of bacteriophage P1 DNA. Mol. Gen. Genet. **153**:311–324.
2. **Bhaskaran, K., V. B. Sinha, and S. S. Iyer.** 1973. Chromosome mobilization in *Vibrio cholerae* (biotype El Tor) mediated by sex factor P. J. Gen. Microbiol. **78**:119–124.
3. **Datta, A., C. D. Parker, J. A. Wohlhieter, and L. S. Baron.** 1973. Isolation and characterization of the fertility factor P of *Vibrio cholerae*. J. Bacteriol. **113**:763–771.
4. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Gerdes, J. C., and W. R. Romig.** 1975. Complete and defective bacteriophages of classical *Vibrio cholerae*: relationship to Kappa type bacteriophage. J. Virol. **15**:1231–1238.
6. **Humphreys, G. O., and T. A. Trautner.** 1981. Maturation of bacteriophage SPP1 DNA: limited precision in the sizing of mature bacteriophage genomes. J. Virol. **37**:832–835.
7. **Imbesi, F., and P. A. Manning.** 1982. Biotype-specific restriction and modification of DNA in *Vibrio cholerae*. J. Clin. Microbiol. **16**:552–554.
8. **Inman, R. B.** 1974. Denaturation mapping of DNA. Methods Enzymol. **29**:451–458.
9. **Jackson, E. N., D. A. Jackson, and R. J. Deans.** 1978. *Eco*RI analysis of bacteriophage P22 DNA packaging. J. Mol. Biol. **118**:365–388.
10. **Johnson, S. R., B. C. S. Liu, and W. R. Romig.** 1981. Auxotrophic mutations induced by *Vibrio cholerae* mutator phage VcA1. FEMS Microbiol. Lett. **11**:13–16.
11. **Johnson, S. R., and W. R. Romig.** 1979. Transposon-facilitated recombination in *Vibrio cholerae*. Mol. Gen. Genet. **170**:93–101.
12. **Johnson, S. R., and W. R. Romig.** 1981. *Vibrio cholerae* conjugative plasmid pSJ15 contains transposable prophage dVcA1. J. Bacteriol. **146**:632–638.
13. **Kleinschmidt, A., and R. K. Zahn.** 1959. Über Desoxiribonucleinsäure. Moleküle in Protein-Mischfilmen. Z. Naturforsch. Teil C **14b**:770–779.
14. **Mekalanos, J. J., S. L. Moseley, J. R. Murphy, and S. Falkow.** 1982. Isolation of enterotoxin structural gene deletion mutations in *Vibrio cholerae* induced by two mutagenic vibriophages. Proc. Natl. Acad. Sci. U.S.A. **79**:151–155.
15. **Morelli, G., M. A. Montenegro, G. Hillenbrandt, E. Scherzinger, and T. A. Trautner.** 1978. The genome of *B. subtilis* phage SPP1: assignment of 5'-3'-polarity to the complementary strands of SPP1 DNA. Mol. Gen. Genet. **164**:93–97.
16. **Morris, C. F., N. K. Sinha, and B. M. Alberts.** 1975. Reconstruction of bacteriophage T4 DNA replication apparatus from

purified components: rolling circle replication following *de novo* chain initiation on a single-stranded circular DNA template. Proc. Natl. Acad. Sci. U.S.A. **72**:4800–4804.

17. **Ogg, J. E., B. J. Ogg, M. B. Shrestha, and L. Poudayl.** 1979. Antigenic changes in *Vibrio cholerae* biotype *eltor* serotype Ogawa after bacteriophage infection. Infect. Immun. **24**:974–978.
18. **Ogg, J. E., M. B. Shrestha, and L. Poudayl.** 1978. Phage-induced changes in *Vibrio cholerae*: serotype and biotype conversions. Infect. Immun. **19**:231–238.
19. **Ogg, J. E., T. L. Timme, and M. M. Alemohammad.** 1981. General transduction in *Vibrio cholerae*. Infect. Immun. **31**:737–741.
20. **Parker, C. D., D. Gauthier, A. Tate, K. Richardson, and W. R. Romig.** 1979. Expanded linkage map of *Vibrio cholerae*. Genetics **91**:197–214.
21. **Philippsen, P., R. A. Kramer, and R. W. Davis.** 1978. Cloning of the yeast ribosomal DNA repeat unit in *Sst*I and *Hin*dIII lambda vectors using genetic and physical size selections. J. Mol. Biol. **123**:371–386.
22. **Ratcliff, S. W., J. Luh, A. T. Ganescan, B. Behrens, R. Thompson, M. A. Montenegro, G. Morelli, and T. A. Trautner.** 1979. The genome of *Bacillus subtilis* bacteriophage SPP1: the arrangement of restriction endonuclease generated fragments. Mol. Gen. Genet. **168**:165–172.
23. **Schnabel, H., W. Zillig, M. Pfäffle, R. Schnabel, H. Michel, and H. Delius.** 1982. *Halobacterium halobium* phage φH. EMBO J. **1**:87–92.