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Fragment Maps of φX-174 Replicative DNA Produced by Restriction Enzymes from *Haemophilus aphirophilus* and *Haemophilus influenzae* H-I

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φX-174 replicative form (RF) DNA was cleaved by restriction enzymes from *Haemophilus aphirophilus* (Hap) and *H. influenzae* (H-I strain Osaka) (HinH). Five fragments were produced by Hap and eight by HinH. The positions of all of the Hap fragments and six of the HinH fragments were mapped on the genome by heteroduplex transfection and DNA-DNA hybridization methods. In addition, fragment sizes of φX-174 RF DNA and S13 RF DNA by Hap and HinH were compared. Considerable differences of the size of the fragments produced from these closely related phage genomes were observed.

Restriction endonucleases which hydrolyze double stranded DNA at specific sites have been isolated from several bacterial strains. These enzymes are particularly useful when specific DNA fragments of φX-174 replicative form DNA (RF DNA) are to be isolated, since the circularity of the DNA may prevent the use of specific mechanical shearing procedures which have been successfully employed in λ genome fragmentation. In fact, enzymes from *Haemophilus influenzae* strain Rd (Hin) (5), *H. aegyptius* (Hae) (16), and *H. parainfluenzae* (Hpa) (13) were shown to cleave the RF DNA at specific sites. The resulting DNA fragments were fractionated and the positions of some of the fragments on the genome were partially deduced (5, 16). It is useful to isolate and characterize a number of restriction enzymes which cleave the φX-174 genome because the combined use of two or more enzymes will produce more restricted fragments of the genome.

In this communication, we wish to describe two other restriction enzymes originally isolated by Takanami's group from *H. aphirophilus* ATCC 19415 (Hap) (20) and *H. influenzae* H-I strain Osaka (HinH) (21) which also cleave the RF DNA. Five fragments were produced by Hap and eight by HinH. The positions of all of the Hap fragments and six of the HinH fragments were mapped on the genome by heteroduplex transfection and DNA-DNA hybridization methods. In addition, we will show the map of φX-174 amber and temperature-sensitive (ts) mutants isolated in our laboratory.

MATERIALS AND METHODS

Restriction enzymes. Hap (AP enzyme) and HinH (HI enzyme) were obtained by the methods of Sugisaki and Takanami (20) and Takanami and Kojo (21), respectively. The initial stage of this investigation was performed using the purified enzymes generously given to us by M. Takanami.

φX-174 RF DNA was isolated as previously described (10). In some cases, RFI DNA (supercoiled closed circular DNA) was isolated from an RFI and RFII (open circular DNA) DNA mixture by isopycnic centrifugation in CsCl with propydium iodide (17). RF DNA was digested by restriction enzymes as specified by Sugisaki and Takanami (20).

Polyacrylamide gel electrophoresis of the fragmented DNA was performed by the method of Bishop et al. (2) except that sodium dodecyl sulfate was omitted from the gels and the tray buffer. Gel length ranged from 16 to 20 cm and the acrylamide concentration from 3 to 4% depending upon the purpose of the experiments. When radioactivity of the fragments was counted, ethylene diacrylate (EDA) (5% of the concentration of acrylamide) was used as the cross-linker. After the run, gels were sliced into 2-mm fractions. The gel fractions were placed in vials with 10 ml of toluene-based scintillation fluid containing 3.5% protosol (New England Nuclear Corp.) and heated at 43 C for 1 h with occasional shaking. After the gels were completely solubilized, the radioactivity was measured by a Beckman LS233 scintillation counter. When the fragments were to be eluted from the gels, *N-N'*methylene bis acrylamide (Bis) was used as a cross-linker (5% of the concentration of acrylamide). After electrophoresis, gels were soaked in water containing 0.5 μg of ethidium bromide per ml for about 30 min and then scanned with a UV light (UVS-12 mineral light, Ultra-Violet Products Co.) in a dark room. The gel regions with fluorescent bands

containing the DNA fragments were excised and minced with a razor blade. The minced gel pieces were suspended in 2 or 3 ml of 0.1× SSC (SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.2) and heated in boiling water for 15 min and quickly cooled in ice water. The gel suspension was then shaken for 24 h in a reciprocal shaker at 37 C. Eluted fragments were stored at −20 C. When small amounts of radioactive fragments were run, and ethidium bromide staining was not applicable (less than 0.01 μg), the entire gel length was sliced into 1-mm fractions and each fraction was heat-treated and shaken as described above. Portions of eluted fragments were counted and peak regions of each fragment were pooled and stored at −20 C. Gels were usually run 7 h at 5 mA per tube for EDA cross-linked gels and 14 to 16 h at 2.5 mA per tube for Bis cross-linked gels.

DNA-DNA hybridization. Hybridization was performed in formamide at room temperature as described previously for RNA-DNA hybridization (9) with some modifications. Fragments derived from Hap enzyme treatment (0.1 to 0.01 μg) were dissolved in 5 ml of 4× SSC and poured onto nitrocellulose membrane filters (Schleicher and Shuel, 26-mm diameter) which had been presoaked in 4× SSC. The solution was allowed to drain through the filters then the filters were washed with 5 ml of 4× SSC. After drying at room temperature overnight, the filters were heated at 75 C in a vacuum oven for 3 h. They were then numbered with a soft pencil, placed separately in tissue culture plates (35 × 10 mm, Falcon 3001), and ^{3}H-labeled fragments derived from Hin H enzyme treatment (10^{-3} to 10^{-4} μg) were added. Final hybridization mixture (1 ml per plate) contained 35% (vol/vol) formamide and 4× SSC. Plates were placed in a sealed box to avoid evaporation and were incubated from 48 to 72 h at room temperature. Filters were then collected in a large petri dish and washed with an excess of 4× SSC several times to eliminate formamide and unhybridized DNA. Excess 4× SSC on filters was drained off onto tissue paper. Filters were then suspended in 10^{-3} M Tris-hydrochloride buffer (pH 9.0). Usually 50 filters were put in 2,000 ml of buffer and stirred on a magnetic stirrer for 15 min. Filters were then drained onto a stack of tissue paper and dried and counted.

φX mutants and bacterial strains. Amber mutants were isolated by the treatment of wild-type phage with nitrous acid (designated by N prefix), hydroxylamine (H), sodium bisulfite (S) (19), and radioactive decay of deoxycytosine incorporated into mature phage DNA (R) (6). All the ts mutants were derived by the treatment with hydroxylamine. Am 3 and am 9 were from R. Sinsheimer's collection. *Escherichia coli* CR63.1, a gift from I. Tessman, was used as the permissive strain for amber mutants of our collection, and HF4704, a gift from R. Sinsheimer, or C BTCC122 was used as the nonpermissive strain. Am 3 and am 9 were grown in HF4714 (a strain from R. Sinsheimer). *E. coli* KT-1 (λ$^-$ sus$^+$ φX-174-resistant) was used for spheroplast formation. The KT strain originally given to us by E. Tessman was lysogenic for λ and was cured of the lysogen in our laboratory.

Genetic crosses. Crosses of amber mutants have been described previously (15). Amber and ts mutants were crossed in either CR63.1 or HF4714 at 33 C, and the wild-type recombinants were scored on C BTCC122 at 43 C. The map distances are defined as previously reported (15).

Isolation of mutant DNA. Mutants were grown under permissive conditions. For ts mutants, C BTCC122 at 30 C was used. The host bacteria were grown to an optical density at 660 nm (OD_{660}) of 0.3 in tryptone broth (Bactotryptone, 10 g, KCl, 5 g per liter) and infected at an MOI of 0.1 after the addition of $MgCl_2$ to 10 mM and $CaCl_2$ to 5 mM. Upon complete lysis, the culture was chilled in ice water and centrifuged at 15,000 rpm for 20 min. The pellet was suspended in 50 mM Na Borate, 3 mM EDTA, and shaken at 4 C overnight. After centrifugation at 12,000 rpm for 15 min, the supernatant was subjected to CsCl density gradient centrifugation. The phage fractions were pooled and dialyzed against 50 mM Na Borate, 3 mM EDTA buffer. Phage preparations containing 5×10^{10} to 3×10^{11} PFU per ml with reversion frequencies lower than 5×10^{-5} were used for DNA extraction. DNA was extracted from the phage suspension at 55 C with phenol saturated with 50 mM Na Borate, then dialyzed against 30 mM Tris-hydrochloride, 50 mM NaCl, 1 mM EDTA (pH 8.0), and stored at −20 C.

Heteroduplex DNA formation. About 20 μg of wild-type RF-DNA labeled with [^{3}H]thymidine (2.6×10^3 counts per min per μg) were digested by Hap or HinH enzyme. Fragments were separated by electrophoresis (3% acrylamide gel, 16-cm length for HinH fragments; 3.5% acrylamide gel, 20 cm for Hap fragments) and sliced into 2-mm fractions. Each slice was suspended in 0.5 ml of 0.1× SSC and the fragments were eluted as described. Fragment regions were detected by counting a portion of each eluate and appropriate fractions were pooled. Recovery of DNA after elution ranged from 40 to 60%. A 10-μliter amount of each fraction was heated at 98 C for 2 min and quickly cooled in ice water. After the addition of 10 μliters of mutant single-stranded DNA and 10 μliters of 2× SSC, the mixture was incubated for 2 to 4 h at 55 C, then overnight at 25 C.

Spheroplast infection. Spheroplasts were made from KT-1 by the method of Guthrie and Sinsheimer (8), modified by E. Tessman (personal communication). Bacteria were grown to OD_{660} = 0.6 in tryptone broth. A 40-ml amount of the culture was centrifuged for 10 min at 5,000 rpm at room temperature. To the pellet, 0.35 ml of 1.5 M sucrose, 0.17 ml of 30% bovine serum albumin, 20 μliters of 2 mg of lysozyme per ml, and 40 μliters of 0.1 M EDTA (pH 8.0) were added in that order. After mixing for about 30 s, 10 ml of PA medium (8) were added and the mixture was left for 15 min at room temperature. A 0.2-ml amount of 10% $MgSO_4$ was then added and the spheroplasts thus formed were stored in an ice water bath. Five or 10 μliters of heteroduplex DNA was suspended in 0.2 ml of 50 mM Tris-hydrochloride, 50 mM NaCl, pH 8.0, and mixed with 0.2 ml of the spheroplast suspension at 35 C (amber mutants) or at 33 C (ts mutants) and

incubated at the appropriate temperature for 20 min; then 1.6 ml of PAM medium (8) was added and incubation was continued for 4 h. Infected spheroplasts were frozen at −70 C overnight. After defrosting, the total number of phages and the number of wild-type phages were assayed by titering under permissive and nonpermissive conditions, respectively. Mutations rescued by hybridized wild-type DNA fragments were expressed by the ratio of the number of wild-type phage to the total number of phage.

RESULTS

Fragments from Hap and HinH enzymes. About 4 μg of uniformly labeled [^{32}P]RF DNA was digested with 10 units of Hap enzyme or 12 units of HinH enzyme for 12 h at 37 C. Enzyme reaction was stopped by adding 10 mM EDTA, then 20% glycerol (final concentration) containing a small amount of bromophenol blue was added and the digest was subjected to electrophoresis as described in Materials and Methods. Profiles of the sliced and solubilized gels are shown in Fig. 1A (Hap) and Fig. 1B (HinH). Hap produced five distinct peaks and HinH fragments were separated into eight peaks. Fragments were numbered as AP 1 to AP 5 and HI 1 to HI 8 in order of decreasing size. Increased enzyme concentration (up to 50 units), or prolonged incubation (72 h) did not change the profiles. Also, when a higher concen-

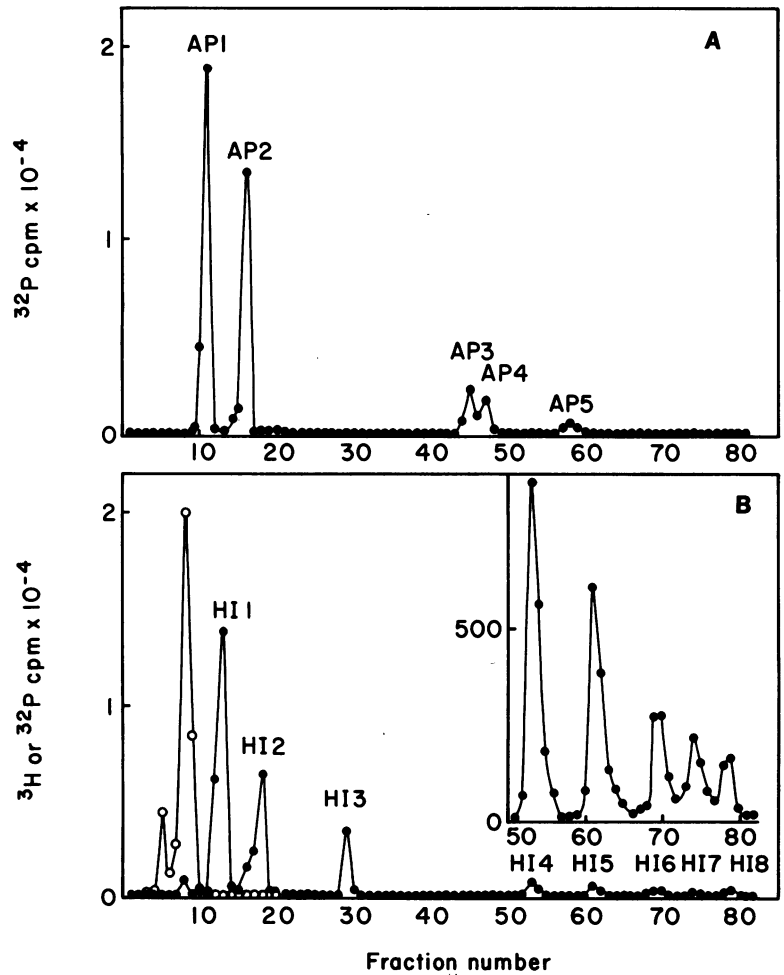


FIG. 1. *Electrophoregrams of ^{32}P-labeled Hap (A, top) and HinH (B, bottom) fragments. EDA cross-linked, 3.5% acrylamide gels (gel length 16 cm) were used as described in Materials and Methods. Bromophenol blue migrated at the HI 6 position. Undigested [^{3}H]thymidine-labeled RF preparation (~4 μg) was run together with the HinH fragments. Symbols: ●, ^{32}P fragments; ○, [^{3}H]RF. HI 4 to HI 8 fragments are also shown in expanded scale in the insert of the HI electrophoregram. In this and the following electrophoregrams, migration of the fragments is from left to right.*

tration of acrylamide (10%) was used, no detectable smaller fragments were found. Therefore, we conclude that the profiles shown in Fig. 1 represent completely digested products of RF DNA with these enzymes. RFI and RFII gave identical patterns (data not shown).

In Fig. 2, ^{32}P-labeled RF digested with Hap and [^{3}H]thymidine-labeled RF treated with HinH were mixed and subjected to electrophoresis to determine the relative electrophoretic mobilities of these two sets of the fragments.

From Fig. 1, we calculated the percentage of total radioactivity contained in each of the gel peaks (Table 1). This should correspond to the percentage of the total genome covered by the fragments in each of the peaks. From Fig. 2 we determined the relative mobilities of each of the peaks. The log of the percentage of the genome contained in each fragment peak is plotted with respect to the relative electrophoretic mobility of the peak in Fig. 3. Smaller molecular weight fragments could be aligned on a straight line, larger ones slightly deviated from this line. Similar observations have been reported by Danna et al. (4). From these results, we concluded that each fragment peak contained only one species from a unique portion of the genome, since, if a peak contained more than one species, it could not be plotted on the straight line observed in Fig. 3.

Mapping fragments by transfection. An amber or ts mutation can be rescued by the infection of spheroplasts by heteroduplex molecules between a mutant DNA and a wild-type DNA fragment which covers the mutated site(s), resulting in the appearance of wild-type progeny. Mutants used in these experiments are mostly from our collection, and only a part of the genetic map has been published (15). Therefore, we summarize here the genetic map of these mutants (Fig. 4). The map is also useful when we correlate the length of the fragment and the results of the heteroduplex transfection.

Recombination frequencies of linked markers within the range of 10^{-4} to 4×10^{-4} units was found to be roughly additive. Orders of very

TABLE 1. *Size of fragments; percent of the total genome*

Fragments	% of total genome	Fragments	% of total genome
AP 1	53.8	HI 1	48.6
AP 2	32.2	HI 2	26.9
AP 3	5.5	HI 3	13.5
AP 4	5.0	HI 4	3.8
AP 5	3.5	HI 5	2.8
		HI 6	1.8
		HI 7	1.5
		HI 8	1.1

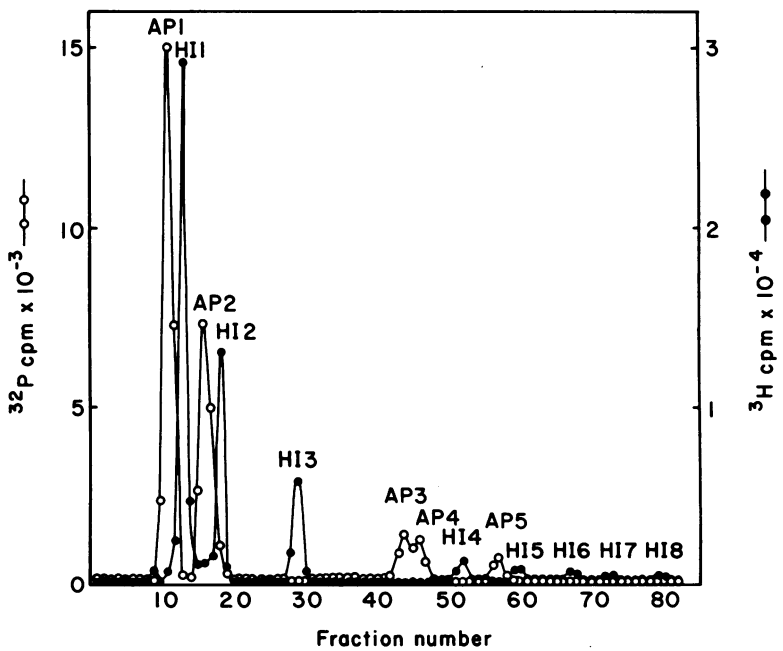


FIG. 2. *Relative positions of Hap and HinH fragments in polyacrylamide gel electrophoresis, ~4 μg of [^{32}P]Hap fragments and 2 μg of [^{3}H]HinH fragments were mixed and analyzed by electrophoresis in EDA cross-linked gel (3.5% acrylamide, 16 cm length).*

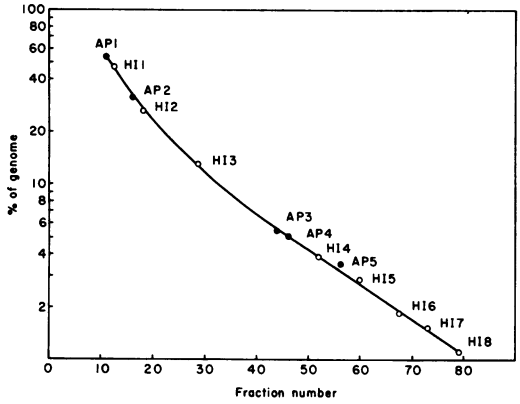


FIG. 3. *Size of fragments produced by Hap or Hin-H enzyme. Results from Table 1 and Fig. 2 were plotted with respect to the position in the gel.*

closely-linked markers (less than 1.0×10^{-4} recombination units) are sometimes ambiguous. The order of the mutants and the distances between markers were mapped on the circular genome by two factor crosses between the appropriate linked markers. The cistron order of A through J has been reported (1). About 60 mutants were crossed and the results are summarized in Fig. 4. Also included are two mutants from Sinsheimer's collection (am 3 and am 9). The total length of the circular genome was found to be 48.1×10^{-4} recombination frequency units by summing up the distance shown in Fig. 4. Benbow et al. (1) reported that intracistronic crosses of the mutants in the A cistron gave much higher recombination frequencies than would be expected on the basis of cistron size determined by the size of the A gene

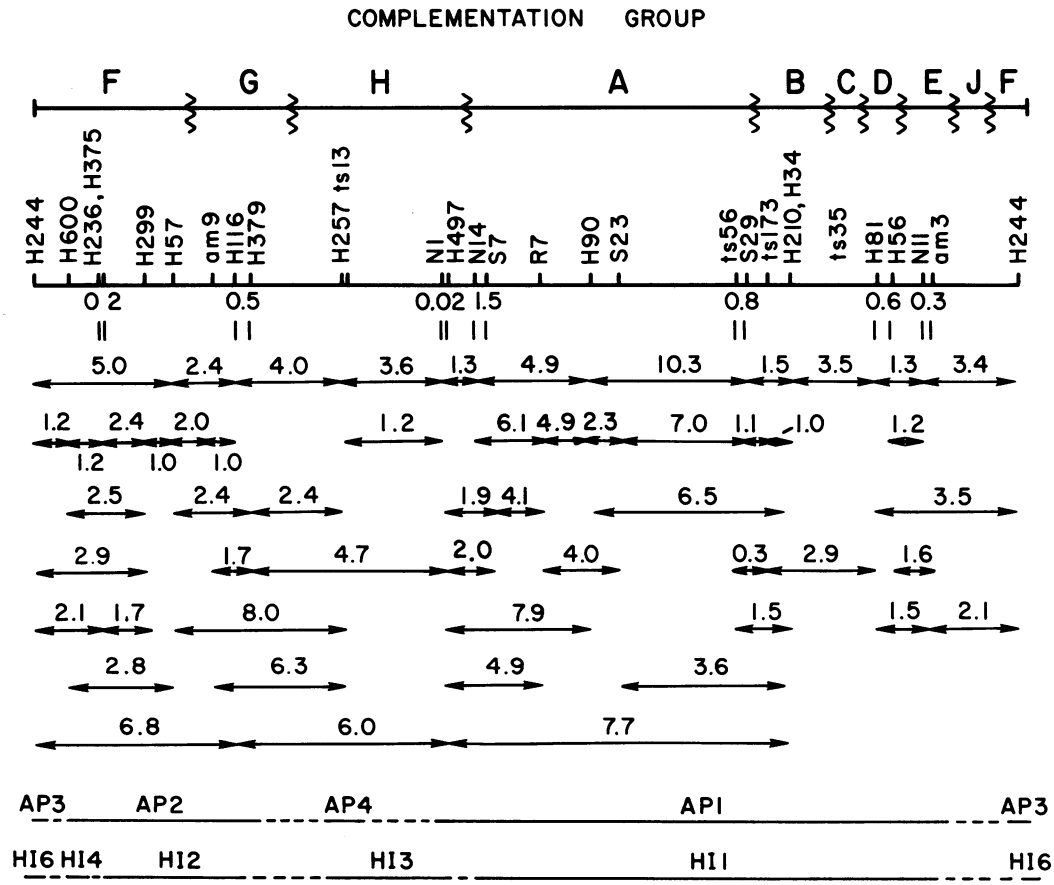


FIG. 4. *Genetic map of the mutants used in heteroduplex infection. Frequency of wild-type recombinants expressed $\times 10^4$. At the bottom of the figure, approximate positions of the fragments determined by heteroduplex infection are shown.*

product (15). We confirmed this observation in this study (see Fig. 4).

Using heteroduplex DNA formed between the mutant DNA and fragmented wild-type DNA, transfection experiments were performed. Results of these experiments are summarized in Table 2 and Fig. 4. For example, the AP 1 fragment which comprises 54% of the genome

TABLE 2. *Heteroduplex transfection*[a]

SS DNA	100 Correspond to	Fragment AP				
		1	2	3	4	5
N14 (A)	2×10^{-1}	100	1.95	0.02	0.04	0.08
S7 (A)	4×10^{-1}	100	0	ND	ND	ND
H90 (A)	1.9×10^{-1}	100	1.98	ND	ND	ND
S29 (A)	8×10^{-2}	100	0.09	ND	ND	ND
H34 (B)	1.7×10^{-1}	100	3.70	ND	ND	ND
H56 (D)	6.2×10^{-2}	100	1.27	0.05	0.06	0.04
H81 (D)	1.1×10^{-1}	100	4.40	0.10	0.18	0
N11 (E)	1×10^{-2}	100	3.20	0.03	ND	ND
am3 (E)	2.2×10^{-3}	100	6.35	0.59	0.11	0.11
H244 (F)	2.5×10^{-2}	0.12	0.12	100	9.20	0.12
H600 (F)	2.0×10^{-1}	0.14	100	ND	ND	ND
H236 (F)		ND	ND	ND	ND	ND
H375 (F)	3.9×10^{-2}	1.15	100	ND	ND	ND
H57 (F)	3.5×10^{-2}	0.31	100	0.09	0.09	0.03
H116 (G)	1.4×10^{-2}	0.15	100	ND	ND	ND
H257 (H)	4.5×10^{-2}	1.90	1.71	3.3	100	3.28
ts13 (H)	2.5×10^{-2}	11.4	3.67	3.26	100	29.7
S8 (H)	5.5×10^{-1}	100	3.09	0.13	0.20	0.02
R4 (H)		ND	ND	ND	ND	ND
N1 (H)	1.5×10^{-1}	100	4.93	0.16	0.03	0.02
H497 (H)	9.1×10^{-1}	100	13.1	2.80	ND	ND

SS DNA	100 Correspond to	Fragment HI						
		1	2	3	4	5	6	7
N14 (A)	4.9×10^{-2}	100	2.20	0.29	0.03	0.03	0.05	0.08
S7 (A)	2.7×10^{-1}	100	2.66	0.24	ND	ND	ND	ND
H90(A)	6.8×10^{-1}	100	1.91	0.16	ND	ND	ND	ND
S29 (A)	2.3×10^{-1}	100	2.48	0.43	ND	ND	ND	ND
H34(B)	2.5×10^{-1}	100	0.60	0.18	0.04	0.08	0.06	0.04
H56 (D)	3.2×10^{-2}	100	5.31	0.47	ND	ND	ND	ND
H81 (D)	7.8×10^{-2}	100	15.4	2.43	0.22	0.02	0	0.42
N11 (E)	7.4×10^{-3}	100	0.88	0.04	ND	ND	ND	ND
am3 (E)	1.5×10^{-2}	100	7.14	0.14	0.07	0.03	0.01	0.01
H244 (F)	2.0×10^{-2}	0.02	0.49	0.41	2.42	10.0	100	0.46
H600 (F)	7.3×10^{-2}	13.4	2.46	0.94	100	0.80	0.12	0.19
H236 (F)	1.9×10^{-1}	13.1	100	0.20	11.1	7.52	9.26	5.26
H375 (F)	5.2×10^{-2}	29.6	100	1.38	2.95	0.48	0.23	0.71
H57 (F)	9.0×10^{-3}	22.2	100	1.22	0.19	0.24	0.11	0.05
H116 (G)	3.7×10^{-2}	12.2	100	0.27	ND	ND	ND	ND
H257 (H)	1.7×10^{-1}	3.68	0.25	100	ND	ND	ND	ND
ts13 (H)	2×10^{-1}	0.06	0.01	100	ND	ND	ND	ND
S8 (H)	1.1×10^{-1}	18.9	0.89	100	0.73	0.13	0.03	0.24
R4 (H)	1.1×10^{-1}	10.2	0.91	100	0.09	0.12	0.04	0.02
N1 (H)	8.7×10^{-2}	28.9	4.85	100	1.51	0.12	6.53	0.24
H497 (H)	7.7×10^{-1}	14.2	3.25	100	ND	ND	ND	ND

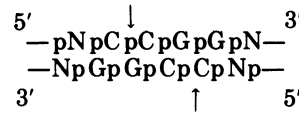
[a] The heteroduplex transfection method is detailed in Materials and Methods. The AP and HI fragments were annealed to mutant single-stranded DNA and the resultant heteroduplex was assayed on spheroplast. The ratio of wild type to total phage yield was determined. The fragments which yielded the highest ratio of the wild type to the total phage are shown as 100. (Actual ratios are shown in the column "100 correspond to".) ND, Not determined.

(Table 1) rescued those mutations from near the C terminal region of H cistron through the entire A, B, C, and D cistrons into at least part of the E gene. The mutations ts13 and H257 which map in the H cistron were rescued by the HI 3 and by the AP 4 fragments. We could not find any mutants which could be rescued by the AP 5, HI 5, or HI 7 fragments. HI 8 was not tested.

Arrangement of fragment position on the genome by DNA-DNA hybridization. Heteroduplex transfection method described in the preceeding section estimated the position of AP 1, AP 2, AP 3, AP 4, HI 1, HI 2, HI 3, HI 4, and HI 6 fragments on the ϕX-174 genome. Unavailability of suitable mutants limited positioning AP 5, HI 5, HI 7, and HI 8. Also, it was desirable to establish more precise positions for the two sets of fragments with respect to each other. Therefore, DNA-DNA hybridization was performed between the AP and HI fragments. Table 3 shows the results of such experiments. AP fragments were fixed on membrane filters and were challenged by HI fragments. Two classes of hybrid formation between HI fragments and AP fragments were observed. One is the HI fragment which hybridizes to only one species of AP fragment. For example, HI 1 hybridizes with AP 1 only. The other set of HI fragments could be hybridized to more than one AP fragment: HI 3 hybridized to AP 1, AP 2, AP 4, and AP 5. The first class contains HI fragments which are completely covered by the appropriate AP fragment, whereas HI fragments in the second class "connect" more than one AP fragment. Knowing the size of the fragments and the relative positions of some of the fragments on the genome, the results in Table 3 provide information determining the positions of all the fragments except for the relative positions of HI 7 and HI 8. These results, showing the physical and genetic maps of the fragments, are summarized in Fig. 5.

DISCUSSION

Sugisaki and Takanami (20) reported that Hap enzyme introduces duplex cleavages on DNA at



The sequence has twofold rotational symmetry. Thus, Hap restricts only a sequence of four nucleotide pairs (CCGG). They also observed that 13 fragments were produced by Hap from fd RFI DNA which has a similar size and base composition to ϕX-174 RF. Upon examining our results, this unique four nucleotide sequence does not exist randomly on the ϕX-174 genome. The AP 1 fragment, which covers more than 50% of the genome does not possess this sequence. The specific sequence for the HinH enzyme has not been determined. However, if we assume that this enzyme requires a specific sequence as do Eco_{RI} (11), Hin (14), and Hap, then this presumed sequence is also not distributed randomly on the ϕX-174 genome. The HI 1 fragment which occupies about 50% of the genome does not contain this sequence. Six out of the eight specific sequence sites were distributed in 10% of the genome which contains the J and the beginning of the F cistron (Fig. 5).

We have almost completed the map of the two sets of fragments. The only ambiguous part is the unknown order of HI 7 and HI 8. These two fragments can be hybridized to AP 3 only. Also, no mutants are available around this region. The accuracy of the fragment maps with respect to the genetic map is rather high. Because the H244 mutation is covered by HI 6, which has only 1.8% of the total genome, the error in the position of the HI fragment map relative to the genetic map can only be about

TABLE 3. *DNA-DNA hybridization between AP fragments and HI fragments*[a]

Fragments	HI-1[b] 6.8×10^3[c]	HI-2 4.4×10^3	HI-3 2.9×10^3	HI-4 1.3×10^3	HI-5 1.2×10^3	HI-6 0.4×10^3	HI-7 0.3×10^3	HI-8 0.25×10^3
AP-1	30	0.8	31	0.3	15	0.7	0.7	0.7
AP-2	0	33	10	34	0.7	26	1.5	5.5
AP-3	0	0	0.45	0.3	23	19	38	17
AP-4	0	0	18	0.2	0.2	0.6	1.0	0.8
AP-5	0	0.5	5	0.2	0.2	1.0	1.0	0

[a] Numbers represent percent of total input counts hybridized. Difference of hybrid formation between particular HI fragments and different AP fragments is not clearly understood; it may reflect a different efficiency of hybrid formation due to base composition and/or length of homology.

[b] HI Fragment.

[c] Counts per minute.

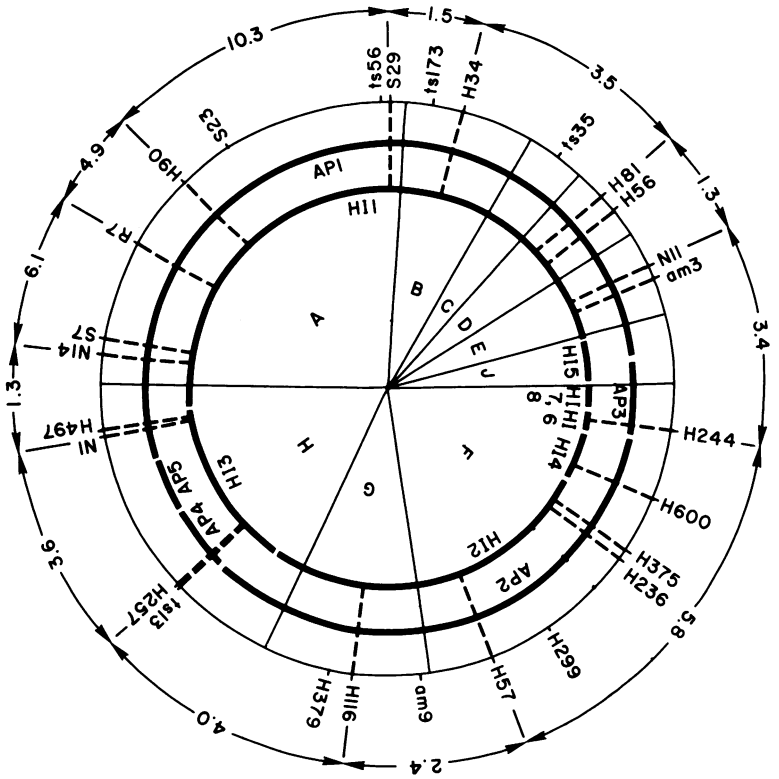


FIG. 5. *Summary of the genetic and fragment maps. The outermost circle represents recombination frequencies (expressed × 10^4) among the representative mutants. The next circle shows the genetic map of the mutants used for heteroduplex transfection experiments. The two inner circles are drawn to represent the physical and genetic positions of the fragments produced by Hap (AP) and HinH (HI) enzymes, respectively. The wedges represent the size of each cistron. This has been determined from measurements of the molecular weight of each cistron product in several SDS-polyacrylamide gel electrophoresis systems by E. Siden and F. Fujimura in our laboratory (manuscript in preparation).*

1.8% of the total genome. Since HI 6 connects AP 3 and AP 2, and AP 3 also covers the H244 mutation, the position of the AP fragment map can also rotate only 1.8% with respect to the genetic map. Therefore, these two fragment maps are constructed as shown in Fig. 5.

The maximum amount that these two fragment maps can slide with regard to each other would be only 1.8% of the total genome. These topological restrictions imposed on the fragment maps and the position of the H244 mutation are useful to examine the accuracy of the two-factor crosses employed in the genetic map construction.

Since the gene A map is more expanded than the physical map when compared to the molecular weight of the gene product (gene A protein) for our analysis we first eliminated this portion of the genetic map (21.7 map units) (assuming that N14 and S29 are very close to the N and C termini, respectively, of the A protein) from the total recombination units (48.1). The H244 mutation was assumed to be at the middle of HI 6 fragment. Starting at H244 and using the recombination units as a guide, each mutant was placed on the fragment map. We were able to place all the mutants examined (except gene A mutants) on the expected fragments. For example, N1 and H497 (the mutations most far away from H244) could be placed on AP 1 and the HI 3 fragments. These indicate that the two-factor crosses are quite accurate. However, if we include the map distance of gene A in this operation some mutants can not be placed on the expected fragment. We speculate that this type of comparison between the fragment map and the genetic map can be applied to other systems in order to detect anomalies in the genetic map (such as gene A).

These two fragment maps are the first to be

completed for ϕX-174. Lee and Sinsheimer have arranged the physical order of Hin, Hpa, and Hae fragments (personal communication). Since these five sets of fragment maps are available, combined use of these enzymes will make it possible to isolate almost any part of the ϕX-174 genome.

APPENDIX

Comparison of ϕX-174 and S13 RF fragments by Hap and HinH enzymes. ϕX-174 and S13, two small icosahedral bacteriophages, were isolated independently (3, 18). Both contain single-stranded circular DNA, and are serologically related (23). In mixed infections they genetically complement (12) and recombine (22). The pattern of phage-coded proteins in SDS-polyacrylamide gels is similar (12), if not identical (7), with respect to the size and the amount of each homologous gene product for these two phages. However, Godson (7) found by heteroduplex formation that, despite these similarities, only 4.7 ± 1.9% of the DNAs from ϕX-174 and S13 is highly homologous, the rest having an overall average base mismatch of 36%. Therefore, such unexpected heterology in the base sequences between these closely related phage genomes would be expected to create different cleavage patterns of the two RF DNAs by restriction enzymes.

ϕX-174 RF DNA was uniformly labeled with [^{32}P]- and S13 RF DNA with [^{3}H]thymidine. The two DNAs were mixed and digested by Hap enzyme or by HinH enzyme and the fragments were analyzed by polyacrylamide gel electrophoresis. The results are shown in Fig. 6. As described, ϕX-174 RF DNA produced five fragments upon Hap enzyme digestion. S13 RF DNA, however, was separated into six peaks (Fig. 6A). When the log of the percent of the genome of each

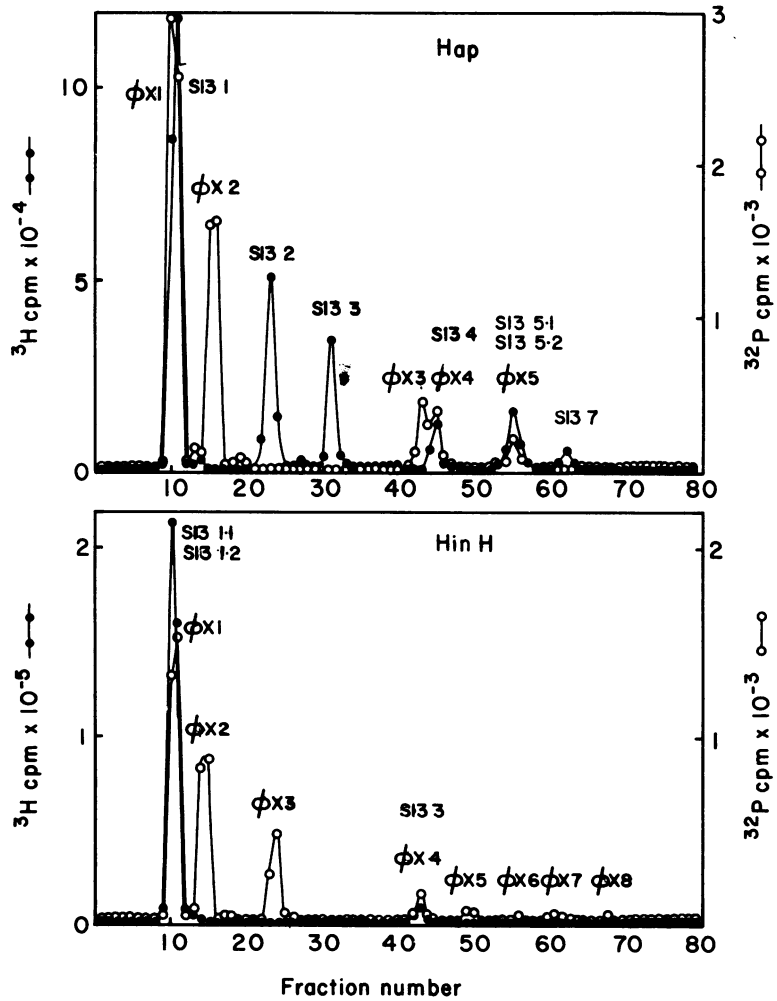


FIG. 6. *Comparison of the fragment sizes of ϕX-174 and S13 RFs produced by Hap enzyme (top) and by HinH enzyme (bottom).*

fragment peak was plotted against the distance of migration, S13 peak 5 (fraction 55) did not fall on a straight line with the other S13 peaks, but it showed that the peak 5 contained two fragments (doublet 5.1 and 5.2). Therefore, S13 RF was cleaved into seven fragments by the Hap enzyme. The S13.1, S13.2, S13.3, and S13.7 peaks did not overlap with any of the φX-174 Hap fragment peaks.

HinH digests of φX-174 RF DNA and S13 RF DNA also showed considerable difference in the gel patterns (Fig. 6B). φX-174 RF DNA was separated in eight singlet peaks, whereas S13 RF DNA gave only two peaks. Summation of the radioactive counts in these two peaks showed that the large and small peaks contained 95 and 5% of the total counts, respectively. The smaller peak of S13 overlapped with φX-174 peak 4 which contained 5% of the total genome. The larger S13 fragment peak comigrated very closely with φX-174 HI 1 which contained 48% of the total genome. From these figures we conclude that the larger S13 peak must contain two fragments (S13 1.1 and S13 1.2) which are almost the same size. Thus, the HinH enzyme cleaved S13 RF DNA into three fragments.

The specific sequence which the HinH enzyme can recognize has not been determined. Sugisaki and Takanami (20) reported that the Hap enzyme cleaves duplex DNA at a CCGG sequence. If one of these four nucleotides is replaced with another nucleotide, the new sequence will be protected from cleavage by Hap enzyme. On the contrary, if, for example, a XCGG sequence (X being A, T, or G) is converted to CCGG, it creates a new site for the enzyme cleavage. Godson's finding that φX-174 and S13 sequences have an overall average 36% mismatch is well reflected in the differences in these two sets of fragment patterns.

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