Proc. Nat. Acad. Sci. USA
Vol. 72, No. 11, pp. 4243–4247, November 1975
Biochemistry

Anatomy of herpes simplex virus DNA: Evidence for four populations of molecules that differ in the relative orientations of their long and short components*

(internal duplications/restriction enzymes/molar ratios/terminal fragments/DNA fragment maps)

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Communicated by Albert B. Sabin, July 28, 1975

ABSTRACT **Intact DNA molecules extracted from HSV-1 (herpes simplex virus 1, human herpes virus 1) strain MP virions have a molecular weight of approximately 97×10^6, but cleavage with the *Hin*III restriction enzyme yields fourteen fragments with summed molecular weights of 160×10^6. Six "major" fragments occur once in every molecule in the population and account for 60% of the genetic information. Four "minor" fragments are present in amounts equivalent to one copy for every two genomes (0.5 molar ratio) and the other four occur only once in every four molecules (0.25 molar ratio). The minor fragments can be arranged into four equimolar sets, each with summed molecular weights that account for the remaining 40% of the genome. Treatment with lambda 5′ exonuclease revealed that all molecules contain 0.5 molar ratio fragments at both termini. These observations and the results of similar analyses of the *Eco*RI and double *Hin*III/*Eco*RI digests indicate that there are four distinct structural forms of HSV DNA which differ only in the relative orientations of two subregions, designated *L* and *S*. The *L* and *S* segments consist of 82 and 18% of the sequences, respectively, and each has inverted terminally redundant regions that correspond to the internal duplications observed by electron microscopy. The DNA from other strains of HSV-1 and 2 also consists of equal proportions of all four possible permutations of the *L* and *S* segments. These unusual features of HSV DNA molecules have novel implications with regard to the genetic map and the mode of replication and evolution of herpes simplex viruses.**

Electron microscopic studies have shown that herpes simplex virus 1, human herpes virus 1 (HSV-1) DNA molecules contain internal inverted duplications of their terminal sequences (1, 2). Measurements on "double-looped" structures formed by intact single-strands after self-annealing indicate that 6.0% of the sequences from the left end (designated *ab*) and 4.3% from the right end (designated *ca*) have adjacent inverted complements (*baac*) located internally in the same strand (see Fig. 1a). Therefore, HSV DNA molecules can be envisaged as consisting of two segments, designated *L* (82% of the sequences) and *S* (18%), each containing unique sequences bounded by a large inverted terminal redundancy, i.e., (*ab...l...ba*) and (*ac...s...ca*). The duplicated *b* and *c* regions were shown to have little if any homology by partial denaturation mapping (2); however, the *a* regions, at the extreme left and right ends of the molecule, represent a noninverted terminal redundancy of less than 1% of the sequences (Wadsworth, Hayward, and Roizman, in preparation).

In another paper in this series (4), we reported evidence for limited heterogeneity in the positions of restriction enzyme cleavage sites among the DNA molecules extracted from purified virons. Digests of HSV-1 and HSV-2 (herpes simplex virus 2, human herpes virus 2) DNAs with either the *Hin*III, *Eco*RI, or *Hpa*I enzymes all yielded constant and reproducible patterns of minor components, irrespective of the strain, multiplicity of infection, or passaging history of the parent virus stocks. Moreover, the summed molecular weights of all major and minor fragments greatly exceeded the known molecular weight of an intact HSV DNA molecule (95 to 100×10^6; ref. 5) and the kinetic complexity of HSV DNA preparations (95×10^6; ref. 6). The tandemly repetitious DNA of high buoyant density that accumulates during serial passaging at high multiplicity (7, 8) was not present in these DNA preparations (4).

We have argued previously that the heterogeneity probably reflects structural rearrangements among individual molecules rather than differences in their genetic content

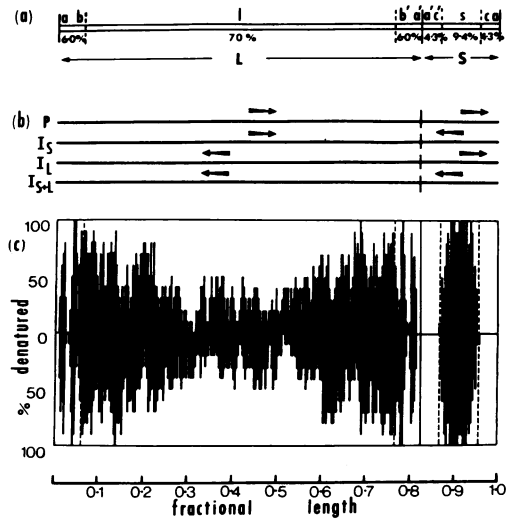

FIG. 1. Diagrammatic representation of the structure of HSV-1 DNA. (a) The regions of HSV-1(F1) identified by electron microscopy (2). The sizes are given as percentages of total length. *a*, terminally redundant regions; *b* and *c*, duplicated sequences unique to the *L* and *S* segments, respectively. (b) The four different permuted forms of HSV-DNA molecules that arise if the *L* and *S* segments have randomly inverted orientations: P, parental or prototype structures; I_S, inverted *S* segment; I_L, inverted *L* segment; I_{S+L}, both *L* and *S* segments inverted. (c) Upper section: histogram of (G+C)-rich(native) and (A+T)-rich(single-stranded) regions constructed from partial denaturation mapping of 10 molecules of HSV-1(Justin) DNA (2). All molecules were oriented with their *S* segments to the right and analyzed in three separate sections to maximize alignments within the *ab*, *ba*, and *S* regions. Lower section: the same histogram with both the *L* and *S* segments inverted right to left to demonstrate symmetrical and mirror-image features.

Abbreviations: HSV-1, herpes simplex virus 1, human herpes virus 1; HSV-2, herpes simplex virus 2, human herpes virus 2; *Hin*III, endoR. *Hin*dIII (restriction endonuclease from *H. influenzae* strain Rd); *Eco*RI, endoR. *Eco*R_1 I (restriction endonuclease from *E. coli* strain RY13).

*This is the fourth paper in a series; paper no. 3 is ref. 8. A preliminary account of some of this work has been presented (ref. 17).

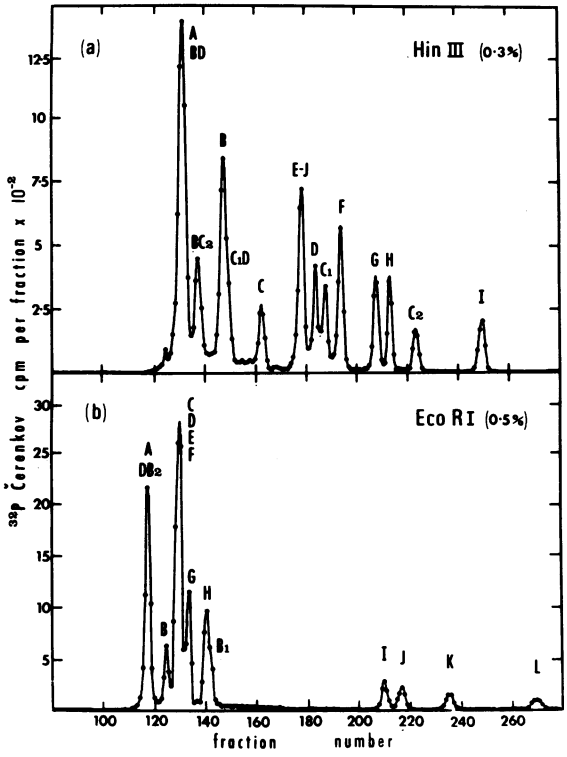

FIG. 2. ^{32}P-Radioactivity profiles of electrophoretically separated fragments from limit digests of HSV-1(MP) DNA with (a) *Hin*III and (b) *Eco*RI enzymes. Gel electrophoresis of digested ^{32}P-labeled HSV-1(MP) DNA was carried out in 1 × 30 cm gels of (a) 0.3% and (b) 0.5% agarose at 1.6 V/cm and 4° in Tris·phosphate buffer (11) for 36 hr. The radioactivity from each of 300 fractions (1 mm gel slices) was measured in toluene scintillation fluid (4). See *text* for discussion.

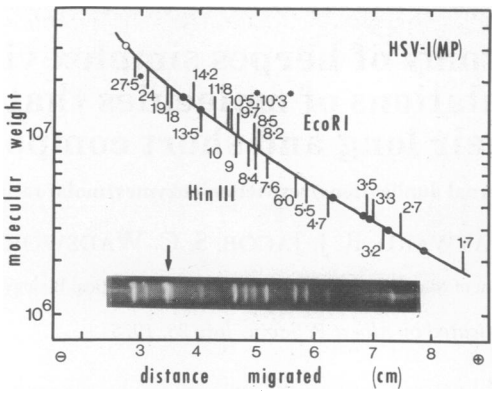

FIG. 3. Molecular weights of the *Hin*III and *Eco*RI fragments. Mixtures of unlabeled fragments from HSV-1(MP) DNA and reference *Eco*RI fragments from λ cI_{857} DNA were subjected to electrophoresis through 0.3% agarose gels (1 × 18 cm) and stained with ethidium bromide (see also Fig. 4, gels 7 and 8). The distances migrated by reference species were measured and used to construct the standard curve for molecular weight versus mobility: intact linear λ cI_{857} DNA 31 × 10^6 (○); *Eco*RI fragments from λ cI_{857} DNA (12, 13), A+F (15.8 × 10^6), A (13.7 × 10^6), B (4.7 × 10^6), C (3.7 × 10^6), D (3.5 × 10^6), E (3.0 × 10^6), and F (2.1 × 10^6) (●).

*Two species present at each of these positions. Inset: ethidium bromide-stained *Hin*III fragments of HSV-1(MP) DNA after electrophoretic separation in 0.3% agarose gels. Arrow indicates the double band of 18 to 19 × 10^6 molecular weight (fragments B and C1D).

(4). For example, the *L* and *S* segments might be inverted relative to each other, giving rise to four different structural forms of HSV DNA as was suggested on theoretical grounds by Sheldrick and Berthelot (1). In the present communication we report evidence confirming the existence of four inverted permutations of the HSV DNA sequences and present a model for the distribution of restriction enzyme cleavage sites that explains the observed heterogeneity in the DNA fragment patterns.

MATERIALS AND METHODS

Preparation of ^{32}P-Labeled HSV DNA. Uninfected HEp-2 cells were washed with several changes of phosphate-free EMEM (Eagle's minimal essential medium) 2% serum followed by incubation for 8 hr in similar medium containing 15 μCi of ^{32}P-labeled orthophosphate per ml. The cultures were then infected with HSV-1(MP) virus (4) at a multiplicity of 10 plaque-forming units per cell and incubated at 34° for 36 hr in the same ^{32}P-containing medium. Enveloped nucleocapsids were purified from cytoplasmic extracts by centrifugation through Dextran T-10 density gradients (9) and the DNA was extracted as described previously (4).

Enzymes. The procedures for purification of the *Hin*III and *Eco*RI restriction enzymes and the conditions for limit digestion of HSV DNA were reported elsewhere (4). Lambda 5′ exonuclease was purified through to the DEAE-cellulose stage from the *Escherichia coli* 1100 (λ t11) lysogen (10). Nearly 50% of native HSV DNA molecules formed circles after solubilization of less than 1% of the terminal sequences with this enzyme preparation. DNA at 20 μg/ml was incubated at 24° with substrate saturating amounts of enzyme in 0.067 M glycine·KOH (pH 9.6), 10 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 30 μg of bovine serum albumin per ml, followed by 3 min at 65° to inactivate the exonuclease. The samples were then dialyzed and subjected to limit digestion with *Hin*III or *Eco*RI.

Agarose Gel Electrophoresis. Apart from the high resolution ^{32}P-profiles which were obtained in long cylindrical gels of 1 × 30 cm, all procedures were performed exactly as described previously (4, 11). To extract *Hin*III DNA fragments from 0.3% gels for redigestion with *Eco*RI enzyme, the gels containing ^{32}P-labeled DNA were cut into 1 mm slices for measurement of radioactivity by the Cerenkov procedure. The appropriate slices were dissolved by shaking in 3 volumes of saturated KI solution in 0.01 M Tris·HCl (pH 8) at 37° for 15 min. After dialysis, the agarose was pelleted by centrifugation (10,000 × *g* for 20 min) and unlabeled carrier DNA was added to the supernatant. The DNA was digested with *Eco*RI and the resulting fragments were separated by electrophoresis through 0.5% gels and located by autoradiography.

RESULTS

Molar ratios of *Hin*III fragments

Fig. 2a shows the radioactivity profile of electrophoretically separated *Hin*III fragments from a limit digest of ^{32}P-labeled HSV-1(MP) DNA. High resolution analysis in 0.3% agarose gels gave 14 bands (see inset of Fig. 3), but two of these (18 × 10^6 and 19 × 10^6 molecular weight) formed a single peak in the profile. Measurements of the molecular weights (Fig. 3) and relative molar concentrations of individual fragments are summarized in Table 1. Using a value of 97 × 10^6 for the molecular weight of an intact HSV-1(MP) DNA molecule (2, 5), we conclude that the *Hin*III digest consists of six "major" fragments present at an average of one copy every genome, four "minor" fragments at close to 0.5 molar ratio relative to major fragments (i.e., one copy

Table 1. Nomenclature, molecular weights, and molar ratios of the *Hin*III and *Eco*RI fragments from HSV-1(MP) DNA

*Hin*III				*Eco*RI products[d] (mol. wt. × 10^{-6})	*Eco*RI		
	Molecular weights[a] (× 10^{-6})	% of ^{32}P[b]	Molar ratio[c]		Molecular weights[a] (× 10^{-6})	% of ^{32}P[b]	Molar ratio[c]
A	27.5		(0.86)	10.3,3.7,3.7,3.3	A 14.2		(0.92)
		30.2	1.07	2.7,1.7,(1.2)		20.2	1.38
BD	27.5		(0.21)	14.2,7.3, 5.4	DB2 (14.2)		(0.46)
BC2	24	5.1	0.21	14.2,7.3,(1.4)	B 11.8	6.5	0.53
B	19		(0.51)	10.5,7.3	C 10.5		(1.02)
		14.6	0.76				
C1D	18		(0.25)	11.8,5.4	D (10.5)		(0.51)
						38.1	3.55
C	13.5	3.6	0.26	11.8, (1.4)	E 10.3		(1.01)
E-J	10.0	10.7	1.04	10.0	F 10.3		(1.01)
D	9.0	5.5	0.59	5.4,3.5	G 9.7	10.7	1.07
C1	8.4	4.6	0.53	8.2	H 8.5		(1.08)
						13.9	1.61
F	7.6	7.7	0.98	6.7, (1.1)	B1 8.2		(0.53)
G	6.0	6.2	1.00	6.0	I 3.5	3.3	0.92
H	5.5	5.5	0.97	4.2, (1.5)	J 3.3	2.8	0.82
C2	4.7	2.6	0.54	3.5, (1.4)	K 2.7	2.2	0.79
I	3.2	3.5	1.05	3.2	L 1.7	1.4	0.81
					M 1.2	—	—

[a] Minor fragments with molecular weights given in parentheses are "hidden" species whose existence was deduced indirectly from molar ratios analyses and the results of double *Hin*III/*Eco*RI digests, etc. The *Hin*III-(E-J) species of HSV-1(MP) represents the fused products of fragments E and J from other strains such as HSV-1(F1) (4).

[b] The measured ^{32}P-radioactivity for each fragment (average from two independent DNA samples) is expressed as a percentage of the total ^{32}P-radioactivity present in all peaks in the profile.

[c] Molar ratios were calculated by dividing the % of ^{32}P-column by the fractional molecular weight (i.e., molecular weight of fragment/molecular weight of intact HSV DNA). Estimates for the individual species within multiple fragment peaks are given in parentheses.

[d] Experimental details are given in *Materials and Methods*.

every two genomes), and four minor fragments present in amounts equivalent to one copy for every four parental DNA molecules (0.25 molar ratio). The existence of "hidden" DNA species in the 27.5 × 10^6 and 18 to 19 × 10^6 molecular weight peaks was confirmed by redigestion of isolated *Hin*III fragment DNA with the *Eco*RI enzyme (Table 1). In both cases the products had summed molecular weights equal to twice that of the original DNA fragments and could be arranged into two sets with different molar concentrations (assumed to be in ratios of 4:1 and 2:1, respectively). The major fragments (total molecular weight 59.7 × 10^6) accounted for 58% of the total mass of HSV DNA and the 0.5 and 0.25 molar ratio species each contributed approximately 21%.

Identification of terminal fragments

The pattern of minor fragments described above can be explained if there are four different structural forms of HSV DNA that occur in equal proportions and differ only in the orientations of their *L* and *S* segments relative to one another (as diagramed in Fig. 1b). Provided that the *Hin*III enzyme does not cleave within the duplicated *ab* sequences, molecules with leftward-oriented *L* segments should yield a different-sized terminal fragment from those of molecules with rightward-oriented *L* segments and both fragments should have molar ratios of 0.5. Similarly, two different 0.5 molar ratio terminal fragments should be obtained from the *S* side of the molecules, if there are no cleavage sites within the *ac* regions.

To identify the terminal fragments in HSV-1(MP) DNA we compared the electrophoretic profiles of *Hin*III digests from untreated and lambda 5′ exonuclease digested DNAs. As shown in Fig. 4, gels 1–3, removal of either 6 or 14% of the nucleotides (mostly from the 5′ ends of the molecules) resulted in the complete disappearance from the gel profiles of all four of the 0.5 molar ratio fragments, whereas the relative proportions of all other species, including the 0.25 molar ratio fragments, were unaffected. We can exclude the possibility that some internal fragments might have been exposed by digestion past the first *Hin*III site(s) because the concentrations of all four fragments were also considerably diminished after a much shorter incubation resulting in only 2% of the DNA being rendered acid-soluble (not shown). These results indicate that all molecules in the HSV DNA population yield *Hin*III fragments of the 0.5 molar ratio type from both their left and right termini as predicted above.

Arrangement of 0.5 and 0.25 molar ratio *Hin*III fragments

In the *Hin*III fragment maps shown in Fig. 5a, all molecules contain a total of nine fragments: the six major fragments whose sizes are unaffected by changes in orientations, two 0.5 molar ratio terminal fragments containing *ab* or *ca* sequences, and one 0.25 molar ratio fragment spanning the internal (*baac*) duplications. Each of the four possible molecular arrangements contains a different set of three minor fragments whose combined molecular weights account for the remaining 40% of the genome (approximately 41 × 10^6). We have designated the fragments in the prototype structure (P) shown in the top line in Fig. 5a as A, B, C, etc., in order of decreasing size. Of the three minor fragments involved here, B (19 × 10^6) is too large to fit into the *S* side and must be the terminal fragment on the *L* side, leaving D (9.0 × 10^6) to be the *S* side terminal fragment. Minor frag-

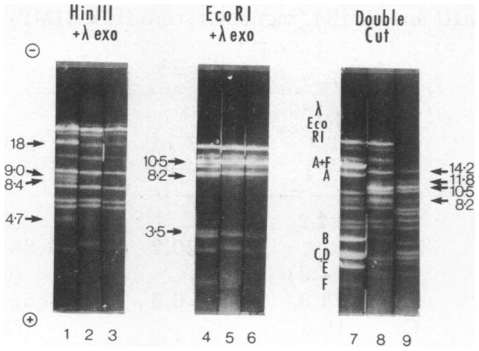

FIG. 4. Identification of terminal fragments by lambda exonuclease treatment and double digests with the *Hin*III and *Eco*RI enzymes. The gels were stained with ethidium bromide after electrophoretic separation of fragments from limit digests of viral DNA. Gels *1–3*, HSV-1(MP) DNA, *Hin*III fragments in 0.3% agarose; gels *4–6*, HSV-1(MP) DNA *Eco*RI fragments in 0.5% agarose. Gels *1* and *4*, controls without exonuclease; gels *2* and *5*, samples preincubated with λ exonuclease for 15 min (approximately 6% digestion as measured by % acid soluble ^{3}H-radioactivity); gels *3* and *6*, 50-min incubations (14% digestion). Positions of terminal fragments are indicated by arrows. Gels *7–9*, fragments from HSV-1(72-43) DNA (4) in 0.5% agarose. Gel *7*, mixture of *Hin*III fragments comigrated with reference *Eco*RI fragments from λ cI_{857}. [The *Eco*RI-(A+F) species arises by annealing of complementary single-stranded termini in 60–70% of the λ molecules, ref. 12.] Gel *8*, *Hin*III fragments mixed with *Eco*RI fragments after separate digestion. Gel *9*, double digest with a combination of the *Hin*III and *Eco*RI enzymes. Remaining 0.5 molar ratio fragments are indicated by arrows.

ment C (13.5 × 10^6, 0.25 molar ratio) covers the internal *baac* region. When the *L* and/or *S* segments invert relative to each other, this *Hin*III C (or C1C2) fragment is in effect split apart at the junction of the two segments; the C1 and/or C2 sequences are shifted out to the *L* and *S* termini, respectively, whereas fragments B and/or D are brought internally and fused with one or other of the terminal fragments from the opposite segment. C1 and C2 correspond to the two remaining 0.5 molar ratio terminal fragments of 8.4 and 4.7 × 10^6 molecular weight, and the 0.25 molar ratio fragments of 17, 24, and 27.5 × 10^6 fit closely to the expected sizes for the fragments C1D (8.4 + 9.0 × 10^6), BC2 (19 + 4.7 × 10^6), and BD (19 + 9.0 × 10^6).

*Eco*RI cleaves within redundant *ac* sequences

Only two minor fragments (11.8 and 8.2 × 10^6) are directly visible in the fragment pattern obtained after *Eco*RI digestion of HSV-1(MP) DNA (Fig. 2b; ref. 14). However, molar ratios analysis (Table 1) indicates that there are probably 11 major fragments (totaling 77 × 10^6) and four minor species all with molar ratios close to 0.5. The 14.2 × 10^6 minor species can be observed directly in the *Eco*RI profile from HSV-1(F1) DNA in which the comigrating major fragment *Eco*RI-A has moved to a different position as the species *Eco*RI-(A-J) (see Fig. 3 of ref. 4). A reduction in the number of minor fragments from eight to four would be expected to occur if the molecules contain symmetrically placed *Eco*RI cleavage sites within either the *b* or *c* sequences. In this case a major fragment with a molecular weight less than that of either the *ab* or *ca* region would occur at one end in all four types of molecules and two of the 0.5 molar ratio fragments would be internal (Fig. 5b). The four 0.5 molar ratio *Eco*RI fragments can be arranged in two ways to complete the full set of genetic information: i.e., D + B (10.5 + 11.8 × 10^6) and B1 + DB2 (8.2 + 14.2 × 10^6).

Experiments involving lambda exonuclease digestion (Fig. 4, gels 4–6) confirmed that the major fragment *Eco*RI-I (3.5 × 10^6) and the 8.2 × 10^6 minor species (*Eco*RI-B1) are terminal fragments. (Presumably, the "hidden" 10.5 × 10^6 species, *Eco*RI-D, which must be the other terminal fragment, is also missing from these profiles.) Since there is insufficient space within *S* to accommodate these two terminal minor fragments, they must both be located within the *L* segment and *Eco*RI-I therefore represents the *S* side terminus.

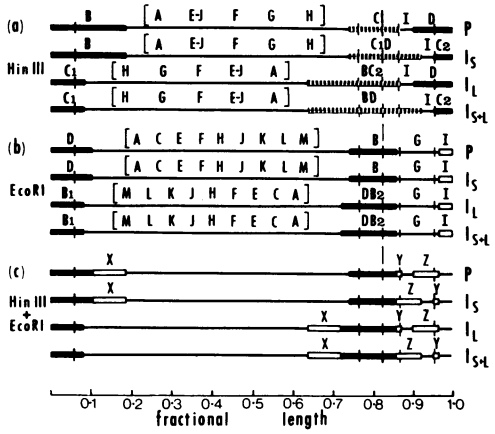

FIG. 5. Maps showing the arrangement of minor fragments in all four structural forms of HSV-1(MP) DNA: (a) *Hin*III, (b) *Eco*RI, and (c) double *Eco*RI/*Hin*III digest. The arrangements labeled P, I_S, I_L, and I_{S+L} correspond to those shown in Fig. 1b. The 0.5 and 0.25 molar ratio fragments are designated by solid bars and striped bars, respectively. Open bars indicate the positions of the terminal major fragment *Eco*RI-I (3.5 × 10^6) in (b) and the sections of minor *Hin*III fragments that are converted to major fragments by the combined action of *Hin*III and *Eco*RI in (c). Fragments labeled X, Y, and Z are common components of the *Eco*RI digests from all isolated *Hin*III fragments that contain "B", "C2", or "D" sequences, respectively (see *text* and Table 1).

Correlation between *Hin*III and *Eco*RI maps

Details of the fragment maps given in Fig. 5 are supported by the following additional evidence. (*1*) Combined cleavage of HSV-1(MP) DNA with the *Eco*RI and *Hin*III enzymes (Fig. 4, gel 9) reduced the total number of minor fragments from eight to four, and furthermore these appear to be the same four species that were observed with *Eco*RI enzyme alone (Fig. 5b). Note that this experiment also provides direct evidence for the existence of the "hidden" *Eco*RI-DB2 species (14.2 × 10^6) in HSV-1(MP) DNA. (*2*) Redigestion of isolated *Hin*III fragments with the *Eco*RI enzyme demonstrates at least three points (see Table 1 and Fig. 5c). (*a*) The *Hin*III-C2 and D fragments both give rise to a 3.5 × 10^6 species and therefore correspond to the *Eco*RI-I end of the molecule. Similarly, the products from *Hin*III-C1 and the *Hin*III-B are at least consistent with their being from the same ends of the molecules as *Eco*RI-B1 and *Eco*RI-D. (*b*) The *L* segment orientation in the *Hin*III structural arrangements labeled P and I_S corresponds to that in the *Eco*RI P and I_S structures. For example, *Hin*III-C contains the *Eco*RI-B sequences and *Hin*III-(C1D + B) DNA gives rise to both *Eco*RI-B and *Eco*RI-D. Similar arguments apply to the two I_L + I_{S+L} structures. (*c*) All *Hin*III fragments suspected of containing "B" sequences (B, BC2, and BD) give rise to a new fragment of 7.3 × 10^6 (X in Fig. 5c) after digestion with *Eco*RI, indicating that they probably do indeed contain common sequences. Similarly, all *Hin*III species which we have suggested contain "D" sequences (D,

C1D, and BD) yield a species of 5.4×10^6 (Z) when further cleaved with *Eco*RI.

Cleavage sites within the *S* segment

The total molecular weight of the *S* side of HSV-1(F1) DNA was estimated at just over 17.2×10^6 by electron microscopy (2). Fragments *Hin*III-C2 and D of HSV-1(F1) DNA together contribute only 14.2×10^6 (4); therefore, we suggest that the 3.2×10^6 species (*Hin*III-I) is located in this gap in the middle of the *S* segment. [Note that *Hin*III-C2 and D of HSV-1(MP) are smaller than their counterparts in HSV-1(F1) by approximately 0.5×10^6.] Evidence supporting the idea that *Hin*III-C2 and I are adjacent comes from the *Hin*III fragment profiles of the HSV-1 (Justin) strain in which the cleavage site between I and C2 appears to have been deleted and bands equivalent to I, C2, C, and DC2 are all missing or displaced (4). In the *Eco*RI map the symmetrical sites at 3.5×10^6 from each end of *S* leave room for an internal fragment of approximately 10×10^6 which is probably *Eco*RI-G, because after relatively long digestions with lambda exonuclease this species was partially lost from the *Eco*RI profiles (Fig. 4, gels 5 and 6).

Correlation with partial denaturation map

Measurements of the amount of [^{3}H]thymidine incorporated into the different fragments (not shown) indicate that the *Hin*III-C2 and *Eco*RI-I fragments have much higher than average (G+C)-base compositions (estimated at 74 and 78 moles % (G+C), respectively, relative to 67% for the intact HSV DNA (5). This result is consistent with the partial denaturation data (ref. 2 and Fig. 1c) which show long (G+C)-rich stretches at the ends of the *S* segment. The histogram given in Fig. 1c indicates that the partial denaturation profiles of both the *L* and *S* segments (even in a very limited sample) have the symmetrical and mirror-image features expected from a population containing equal numbers of leftward- and rightward-oriented *L* and *S* segments.

DISCUSSION

Studies on the *Hin*III and *Eco*RI fragments from HSV-1(MP) DNA indicate that four kinds of HSV DNA molecules occur in the virions in approximately equal proportions. These molecules differ only in the relative orientations of two subregions corresponding to the *L* and *S* segments defined by electron microscopy. The heterogeneity observed among the fragments from many other strains of both HSV-1 and HSV-2 DNAs (4), and, also with the *Hpa*I enzyme, can similarly be explained by the existence of four inverted permutations of the nucleotide sequences. (In HSV-2 DNAs, the *Eco*RI enzyme gives the characteristic eight minor fragments with 0.5 and 0.25 molar ratios, whereas *Hin*III yields a four minor fragment pattern.)

We can envisage at least two possible mechanisms for generating inverted permutations in HSV DNA. As Sheldrick and Berthelot have pointed out (1) the outcome of intramolecular reciprocal recombination events between the homologous *ab* and *ba* or *ac* and *ca* sequences would be simple inversions of all sequences between them, irrespective of the nature of the parent replicating structures (linear monomers, circles, or concatemers). The situation can be considered somewhat analogous to that of the "G" region in phage Mu DNA (15). An alternative "reassortment" mechanism might involve physical separation of the *L* and *S* segments, perhaps through cleavage at the internal *aa* region. Since the terminal sequences at either end of each segment should be indistinguishable, the *L* and *S* segments could rejoin in any orientation relative to one another.

We wish to comment on several possible implications of these novel structural features of HSV DNA. (*1*) Although mutations within the *L* and *S* segments map in a linear fashion, all crosses between markers in *L* and *S* would be expected to give maximal recombination frequencies, and therefore the genetic map for the entire HSV genome should consist of two separate linkage groups that cannot be oriented relative to one another. (*2*) Assuming that all four molecular forms are infectious, the transcriptional program of the virus (position of promotors, polarity, etc.) must be organized in such a way that it can tolerate these large rearrangements of sequences. Furthermore, analysis of transcription will be complicated because intact HSV DNA must contain eight different kinds of single-strands with various combinations of the *l* and *r* strand sequences. (*3*) Despite a 50% sequence divergence (16), the inverted terminal repetitions that demarcate the boundaries of *L* and *S* have been conserved in both HSV-1 and HSV-2 DNAs. Therefore, we suggest that the potential for functioning as two independent "replicons" may have some as yet unknown biological significance. These features could also reflect a convergence from two separate genetic sources during the evolution of herpes simplex viruses.

We thank Marc Rhoades for the *E. coli* 1100 (λ t11) strain and for advice in purifying the lambda exonuclease enzyme. These studies were performed under the auspices of the Chicago Cancer Research Center Project (CA 14599) and were aided by grants from the National Cancer Institute (Public Health Service (CA 08494), the American Cancer Society (VC 103J), and the National Science Foundation (GB38270). R.J.J. is a postdoctoral fellow of the American Cancer Society (PF972) and S.C.W. is a predoctoral trainee of the National Institute of Allergy and Infectious Diseases (Public Health Service Training Grant AI00238).

1. Sheldrick, P. & Berthelot, N. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **39**, 667–678.
2. Wadsworth, S. C., Jacob, R. J. & Roizman, B. (1975) *J. Virol.* **15**, 1487–1497.
3. Grafstrom, R. H., Alwine, J. C., Steinhart, W. L. & Hill, C. S. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **39**, 679–681.
4. Hayward, G. S., Frenkel, N. & Roizman, B. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 1768–1772.
5. Kieff, E. D., Bachenheimer, S. L. & Roizman, B. (1971) *J. Virol.* **8**, 125–132.
6. Frenkel, N. & Roizman, B. (1971) *J. Virol.* **8**, 591–593.
7. Bronson, D. L., Dreesman, G. R., Biswal, N. & Benyesh-Melnick, M. (1973) *Intervirology* **1**, 141–153.
8. Frenkel, N., Jacob, R. J., Honess, R. W., Hayward, G. S., Locker, H. & Roizman, B. (1975) *J. Virol.* **16**, 153–167.
9. Spear, P. G. & Roizman, B. (1972) *J. Virol.* **9**, 143–159.
10. Radding, C. M. (1966) *J. Mol. Biol.* **18**, 235–250.
11. Hayward, G. S. & Smith, M. G. (1972) *J. Mol. Biol.* **63**, 383–395.
12. Allet, B., Jeppesom, P. G. N., Katagiri, K. J. & Delius, H. (1973) *Nature* **241**, 120–123.
13. Thomas, M. & Davis, R. (1975) *J. Mol. Biol.* **91**, 315–328.
14. Skare, J., Summers, W. P. & Summers, W. C. (1975) *J. Virol.* **15**, 726–732.
15. Hsu, M.-T. & Davidson, N. (1974) *Virology* **58**, 229–239.
16. Kieff, E. D., Hoyer, B., Bachenheimer, S. L. & Roizman, B. (1972) *J. Virol.* **9**, 738–745.
17. Roizman, B., Hayward, G. S., Jacob, R. J., Wadsworth, S. C. & Honess, R. W. (1974) "Proc. XI International Cancer Congress, Florence, 1974," in *Chemical and Viral Oncogenesis* (Excerpta Medica International Congress Series no. 350), Vol. 2, pp. 188–198.