Physical Map of the Origin of Defective DNA in Herpes Simplex Virus Type 1 DNA

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The origin of defective DNA (dDNA) of the Patton strain of herpes simplex virus type 1 (HSV-1) was physically mapped with BamHI in the parental DNA. The dDNA obtained from virus passaged at high multiplicities of infection was resistant to cleavage with HindIII, whereas digestion with EcoRI yielded a cluster of fragments 5.4 to 5.7 megadaltons (Mdai) in size. Cleavage with BamHI gave a cluster of fragments 2.6 to 3.2 Mdai in size, plus two homogeneous, comigrating 1-Mdai fragments. One of the latter fragments contained the single EcoRI site approximately 65 base pairs from one end. Hybridization of in vitro labeled dDNA probe to EcoRI, HindIII, BamHI, and Hpa I digests of nondefective HSV-1 DNA demonstrated that, in addition to the S-region terminal repeat, only one end of the S region was involved in the generation of this class of dDNA. Thus, the dDNA probe did not hybridize to either the S region 3.0-Mdai HindIII N fragment or a 3.0-Mdai BamHI fragment of the adjacent 8.7-Mdai HindIII G fragment, but did hybridize to four BamHI fragments of HindIII G (~5.7 Mdai). The cluster of 2.6- to 3.2-Mdai fragments obtained with BamHI digestion of dDNA appears to represent a novel junction between the termination of dDNA adjacent to the 3.0-Mdai BamHI fragment in HindIII G and the 2.0- to 2.3-Mdai BamHI fragment terminal in HSV-1 DNA.

The DNA of herpes simplex virus type 1 (HSV-1) is a linear, double-stranded molecule with a mass of approximately 100 megadaltons (Mdai) (1, 6, 9). Evidence that the structural arrangement of the HSV genome consists of two unique segments, L and S, inverted about and separated by distinct terminally and internally inverted repetitions, was obtained from electron microscopy studies of intact self-annealed single-stranded DNA (14, 21, 22). Additional evidence for this structure was obtained from restriction endonuclease analyses of native DNA employing EcoRI, HindIII, Hpa I, Bam, and Xba I (3, 8, 16, 25). The presence of submolar amounts of restricted DNA fragments led to the conclusion that four possible permutations of the molecule occurred as a result of L and S inversion around the two sets of terminally and internally repeated sequences.

We have examined a portion of the HSV-1 S region and its adjacent repeated sequences, which are related to defective DNA (dDNA). The dDNA of HSV-1, generated by virus passaged at high multiplicities of infection (MOI), has been well characterized (2, 4, 23). For the Justin strain of HSV-1 the dDNA has been shown to consist of tandem repetitions of S-region sequences greater than 5 Mdai in complexity (4, 5). This dDNA is resistant to cleavage with Hsu I and HindIII, suggesting that it originates from a portion of the parental genome lacking these restriction sites. EcoRI cleavage yields two major cleavage fragments, 5.1 and 5.4 Mdai, each arising apparently from two different classes of defective molecules (4, 5). In contrast, dDNA from the KOS strain of HSV-1 yields a large number of fragments when cleaved with EcoRI (23), suggestive of multiple classes of dDNA. In our studies the dDNA of the Patton strain of HSV-1 is shown to be similarly sensitive to cleavage with EcoRI while resistant to HindIII cleavage. Moreover, by using in vitro labeled EcoRI-cleaved dDNA as a probe, sequences related to dDNA are physically mapped in one end of the S region 8.7-Mdai HindIII G fragment (16, 24) with BamHI.

MATERIALS AND METHODS

Cells and viruses. The growth and maintenance of Vero cells (ATCC CCL81) have been described elsewhere (12). HSV-1 strain Patton (obtained from Electronucleonics Inc., Silver Spring, Md.) was propagated in Vero cells at low (0.10 to 0.03 PFU/cell) and at high (greater than 0.1 PFU/cell) MOI. For virus passages, Vero cells growing in T75 flasks (Falcon Plastics, Oxnard, Calif.) were infected and incubated at 37°C until 90 to 100% cytopathic effect occurred. The infected cells were frozen and thawed three times
and used as inoculum for one roller bottle (690 cm², Bellico, Vineland, N.J.) of Vero cells (approximately 10⁶ cells per roller bottle). For subsequent virus passages, the harvest of one roller bottle was used as inoculum for 10 roller bottles. PO is used to designate HSV-1 continually passaged at low MOI, whereas a number indicates the appropriate passage at high MOI.

The isotopically labeled virus, [H]thymidine (20 μCi/ml) (6 Ci/mMol, New England Nuclear, Boston, Mass.) was added at 12 h postinfection, and the virus was harvested when the cells exhibited 90 to 100% cytopathic effect.

Isolation and purification of virus and DNA. The virus was concentrated from the supernatant fluids by polyethylene glycol precipitation and purified by either isopycnic banding in a linear Renografin gradient (12, 15) or sedimentation through a discontinuous sucrose gradient (6). The viral DNA was extracted and purified from HSV as described previously (12). Briefly, virus was lysed with 1% sodium dodecyl sulfate, incubated with 2 mg of autodigested Pronase (Calbiochem, Los Angeles, Calif.) per ml for 3 h at 37°C, and then deproteinized with chloroform-isomyl alcohol (24:1). The aqueous phase containing the DNA was adjusted to a density of 1.717 g/cm³ with crystalline cesium chloride (Schlesinger Chemical Mfg. Corp., Carle Place, N.Y.), and the DNA was centrifuged to equilibrium in a Beckman type 30 or type 65 rotor at 24,000 rpm for 84 h at 20°C. The gradient was harvested by puncturing the tube with a 4 mm intradermic tube, and the DNA was located by radioactivity and/or UV absorption at 260 nm. The DNA-containing fractions were pooled and dialyzed at 4°C against 0.1 x SSC (SSC contained 0.15 M NaCl, 0.015 M sodium citrate, and 1 mM ethylenediaminetetraacetic acid [EDTA]).

Restriction enzyme analyses. The amount of EcoRI (Miles Laboratory, Elkhart, Ind.), HindIII, HpaI, or BamHI (Bethesda Research Laboratories, Bethesda, Md.) needed for complete digestion of 1 to 2 μg of DNA was predetermined by enzyme titration. The reaction mixtures contained: for EcoRI, 100 mM Tris-hydrochloride (pH 7.5)–50 mM NaCl–5 mM MgCl₂; for HindIII, 200 mM Tris-hydrochloride (pH 7.5)–60 mM NaCl–7 mM MgCl₂; for Hpa I, 30 mM Tris-hydrochloride (pH 7.5)–10 mM MgCl₂–6 mM KCl–5 mM 2-mercaptoethanol; for BamHI, 20 mM Tris-hydrochloride (pH 7.5)–7 mM MgCl₂–2 mM 2-mercaptoethanol. Each reaction was stopped by the addition of EDTA (10 mM). The DNA was deproteinized with chloroform-isomyl alcohol (24:1), precipitated with 2 to 3 volumes of ethanol in the presence of 0.3 M sodium acetate, and pelleted at 100,000 × g for 30 min. The pellet was suspended in 0.1× E buffer (E buffer contained 40 mM Tris, 10 mM sodium acetate, and 1 mM EDTA adjusted to pH 7.8 with acetic acid).

Gel electrophoresis and isolation of DNA restriction fragments. Gel electrophoresis was performed as described by Mulder et al. (11) in 30 by 15 by 0.4-cm gel slabs with 0.7% agarose. The DNA fragments were separated by electrophoresis on agarose at 60 V for 40 h at 4°C. The DNA bands were stained with ethidium bromide (0.5 μg/ml) and photographed by using a short-wave UV light source (Mineral Light model C51, Ultra Violet Product, Inc., San Gabriel, Calif.). Molecular weights were estimated relative to adenovirus, HSV-1, or simian virus 40 markers.

Large quantities (100 to 150 μg) of specific HSV-1 or dDNA fragments were obtained after endonuclease restriction and electrophoretic separation on vertical agarose gels. The stained bands were excised from the gel, and the DNA fragments were isolated by the freeze-squeeze method (20). Using this technique, 50 to 70% of the DNA was recovered intact.

Transfer of fragments from agarose gel to nitrocellulose membranes. The procedure for transferring DNA fragments to nitrocellulose membranes has been described by Southern (18). The DNA fragments in agarose gels were denatured by soaking the gel in 0.5 M NaOH–1.5 M NaCl for 30 min at room temperature. This was followed by a distilled water rinse and neutralization with 1.0 M Tris-hydrochloride (pH 7.2)–1.5 M NaCl for 40 min at room temperature. The DNA was transferred onto nitrocellulose membranes using 20× SSC as the eluting buffer. The efficiency of fragment transfer was monitored by UV examination of the agarose gel after restaining with ethidium bromide. The DNA containing nitrocellulose membrane was heated for 2 h at 80°C and pretreated as described (12). Hybridization was performed by placing the membrane in a chamber (described below) and adding the labeled probe in 3x SSC containing 0.5% sodium dodecyl sulfate and 20 μg of yeast tRNA per ml in a minimal volume sufficient to wet the membrane. The conditions for hybridization were 66°C for 20 to 24 h. After hybridization, the membrane was washed exhaustively in 3x SSC, dried, and exposed to Kodak SB54 X-ray film.

A chamber was custom made for each hybridization mixture. Three layers of parafilm were cut 1 mm longer and wider than the nitrocellulose membrane and placed on a siliconized glass plate. The glass surface, in contact with the parafilm, was coated with a thin layer of silicone vacuum grease. The chamber, containing the membrane and hybridization mixture, was covered with another siliconized glass plate and clamped with binder clips. The glass plates were sealed air tight by the action of heat (66°C) on the parafilm and silicon grease.

In vitro labeling of restriction fragments. The conditions for labeling restriction fragments with [32P]-deoxyribonucleotide triphosphates have been described by Maniatis et al. (10). Briefly, the reaction mixture contained in 100 μl: 50 mM Tris-hydrochloride (pH 7.8), 5 mM MgCl₂, 10 mM 2-mercaptoethanol, 50 μg of bovine serum albumin per ml, 0.5 to 1 μg of DNA, 180 pmol of two [32P]dNTP, and 180 pmol of two unlabeled dNTP. The reaction was started by the addition of 2.8 U of Escherichia coli polymerase 1 (Boehringer Mannheim Biochemicals) and 10⁻⁶ μg of DNase I (DPFF, Worthington Biochemical Corp.). After 1 h at 15°C, the reaction was terminated by chloroform-isomyl alcohol (24:1) extraction. The unincorporated nucleotides were removed by gel electrophoresis or ethanol precipitation. The specific activity of the product ranged from 1–10 × 10⁷ cpm/μg.

RESULTS

Generation and characterization of defective HSV DNA. Defective HSV-1 Patton
was generated by passage of the PO virus stock at high MOI. The generation of dDNA was monitored at every fourth passage both by isopycnic centrifugation in CsCl and digestion of extracted viral DNA. The centrifugation analyses showed that with high MOI passages (P8, P12, and P16), new, higher density classes of DNA were detected relative to HSV-1 DNA marker (not shown). The EcoRI cleavage of PO DNA gave the expected profile for HSV-1 passaged at low MOI (Fig. 1) (7, 17). However, the EcoRI analyses of DNA from HSV passaged at high MOI (P4 to P16) showed additional fragments: two distinct minor fragments with molecular weights of 4.3 and 6.5 Mdal (Fig. 1) and a cluster of fragments ranging from 5.4 to 5.7 Mdal. The 4.3- and 6.5-Mdal fragments were not further characterized. Whereas PO HindIII-cleaved fragments were detected in P8 DNA, some of the DNA was resistant to cleavage with HindIII (Fig. 1), as has been found for Justin dDNA (4, 5). The concurrent appearance of new HSV-1 DNA density classes and EcoRI restriction fragments (Fig. 1) after virus passage at high MOI are characteristics of dHSV DNA (4, 23).

The structure of dDNA was examined further by digestion with BamHI (Fig. 2). The disappearance of the 5.4- to 5.7-Mdal EcoRI dDNA fragments after digestion with BamHI and the appearance of a new cluster of fragments, 2.6 to 3.2 Mdal, plus two 1-Mdal fragments (B2 and B3-R), indicated that several BamHI cleavage sites existed in dDNA. In a separate experiment, BamHI cleavage of isolated 5.4- to 5.7-Mdal EcoRI fragments yielded B2, B3-R, and the 2.6- to 3.2-Mdal cluster of fragments, indicating that the latter fragments were responsible for the

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**Fig. 1.** EcoRI restriction enzyme analysis of HSV DNA. DNA from HSV passaged at low (PO) and high (P4, P8, P12, P16) MOI purified by isopycnic centrifugation in cesium chloride was digested with EcoRI or HindIII. The molecular weights of minor, new DNA species (arrows) in the EcoRI digests are 4.3 and 6.5 Mdal, respectively.
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heterogeneity in the EcoRI dDNA. Moreover, the two 1-Mdal dDNA fragments were equivalent in mobility and related by hybridization to two BamHI fragments of PO DNA (B2 and B3) of PO; Fig. 3) (data not shown), indicating that they were normal EcoRI-BamHI cleavage products of PO DNA. Cleavage of the S-region terminal EcoRI K fragment (8, 16, 19, 25) with BamHI (see Fig. 6) yielded the B1 fragment indicated in PO and P8 DNA (Fig. 2). BamHI digestion of P8 DNA produced a single 1-Mdal fragment (B2, B3), while two fragments were detected in the doubly cleaved (EcoRI-BamHI) P8 DNA, suggesting that a single EcoRI site was present on only one of two comigrating 1-Mdal fragments. This was clearly demonstrated using high resolution polyacrylamide gels (Fig. 3) where the mobility of the BamHI B3 fragment increased after EcoRI digestion (B3-R) and a new fragment (R) was detected in 20% acrylamide with an approximate size of 65 base pairs (based on the difference in mobility between B3 and B3-R). The two 1-Mdal BamHI fragments, which comigrated in agarose (Fig. 3), were separated and inverted in mobility with respect to the EcoRI-sensitive fragment (i.e., B3 > B2) in 5% polyacrylamide. This inversion may be due to an effect of polyacrylamide on high guanine-cytidine (G-C)-containing DNA fragments, as has been observed for adenovirus DNA frag-
Determination of the BamHI physical map of dDNA and of PO HindIII G fragment. To map the sequences in HSV-1 DNA related to dDNA, PO DNA cleaved with EcoRI, HindIII or EcoRI-HindIII was annealed by the Southern technique (18) with in vitro $^{32}$P-labeled EcoRI 5.4- to 5.7-Mdal dDNA fragments (Fig. 4). The dDNA probe hybridized to the EcoRI H and K and HindIII F, G, and M fragments (16, 24). These fragments are resolved in agarose as single components and map in the S region or its adjacent repeated sequences. The other dDNA positive fragments could be EcoRI B and C and HindIII B, C, and E, which map in the joint region between S and L (Fig. 5). A possible ambiguity in the identification of latter fragments (Carel Mulder, personal communication). At least two additional BamHI fragments, approximately 0.1 Mdal in size, were also observed in the 20% acrylamide by ethidium bromide staining. These analyses (Fig. 3) were not used for absolute molecular weight determination because of possible G-C effects on the high G-C HSV-1 DNA versus the low G-C SV40 DNA markers.

**Fig. 3.** Polyacrylamide gel electrophoresis of endonuclease-restricted P8 dDNA. High MOI passaged viral DNA was restricted with (A) EcoRI, (B) BamHI, or (C) EcoRI-BamHI and subjected to electrophoresis on a discontinuous 5 and 20% polyacrylamide gel (arrow indicates the interphase). The EcoRI-HpaI cleavage products of SV40 (D) were used as molecular weight standards. The $B_2$ (or $B_2$-R) is identified by its EcoRI sensitivity (Fig. 2).

**Fig. 4.** Determination of the PO HSV restriction fragments related to dHSV DNA. PO DNA was digested with EcoRI, EcoRI-HindIII and HindIII, fractionated by agarose gel electrophoresis (A, C, and E, respectively), and subjected to the Southern technique (18) using $^{32}$P in vitro labeled 5.4- to 5.7-Mdal EcoRI-cleaved dDNA fragments as probe (B, D, and F, respectively). The fragments hybridizing with the dDNA probe are indicated and occur in the S region or in its adjacent repeated units as diagramatically presented in Fig. 5.
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EcoRI C H K
HindIII F N G
R=H 11.5 N G-K 3.5
R=H E N M
R=H G-K N
R=H B H K
R=H N G-K
R=H 13.5 G-K N 3.5

FIG. 5. Schematic presentation of the S region EcoRI, HindIII, and double digest physical maps (according to Skare et al., 16). The EcoRI and HindIII fragments associated with the "joint" and S regions are shown with the four permutations of HSV-1 DNA (—). The doubly cleaved EcoRI-HindIII (R-H) permutations (——) are shown beneath their corresponding permutation. The solid bars above the dotted lines correspond to the fragments that hybridize with the dDNA probe (Fig. 4).

ments could arise because each comigrates with fragments from other regions of the genome (16, 24). However, evidence for dDNA hybridization with only S-region fragments was indicated from the EcoRI-HindIII double digest. The restriction fragment maps of this region (Fig. 5) show the fragments, which hybridized to dDNA in the double digest (R-H), as solid bars above the dotted line map. The largest fragments obtained with the EcoRI-HindIII digest corresponded to EcoRI B (13.5 Mdal) and C (11.5 Mdal) (16) and contained sequences corresponding to the terminal EcoRI K fragment plus a portion of the L region and the L-region repeated sequences. Since none of the L-region EcoRI-HindIII fragments hybridized to the dDNA probe (e.g., EcoRI E and F; 10.2 Mdal through the HindIII K 6-Mdal fragment), the dDNA-related sequences of the Patton strain originated only from the S region. The Justin strain dDNA has also been shown to map in the S region (5). These results also showed that the S region 3.0-Mdal HindIII N fragment did not hybridize with the dDNA probe (as it did with PO probe; data not shown), whereas both the adjacent HindIII G (8.7 Mdal) and the 5.2-Mdal HindIII G minus EcoRI K (G-K) fragment did. HindIII M (4.5 Mdal), containing the 3.5-Mdal EcoRI K fragment (16), also hybridized with the dDNA probe. It was not determined whether the small 1.0-
Mdal fragment generated by EcoRI digestion of HindIII M hybridized with dDNA since this fragment would still contain a portion of the S-region repeated sequence (9, 16, 24).

The hybridization of the 5.4- to 5.7-Mdal dDNA probe with the G-K fragment, but not with HindIII N, suggested that the dDNA was derived from only one end of the S region. To determine the extent of dDNA sequences in the HindIII G fragment, preparative amounts of the latter in the form of EcoRI K (K) and G-K were digested with either BamHI for comparison to the dDNA cleavage (Fig. 2) or Hpa I for the location of the single S region Hpa I site (24). The digests were analyzed by the Southern technique (18) (Fig. 6) by using either in vitro 32P-labeled EcoRI dDNA or in vitro 32P-labeled PO DNA as probes. Partial digestion of G-K with BamHI yielded five fragments (4.4, 3.0, 2.4, 1.2, and 1.0 Mdal) (Fig. 6C and D), whereas complete digestion yielded only the 3.0-, 1.2-, and 1.0-Mdal fragments (PO hybridization pattern Fig. 6E). Partial digestion of K with BamHI yielded 2.0- to 2.3-Mdal fragments, which correspond in mobility to the B1 fragment indicated in the BamHI digests of PO and P8 DNA (Fig. 2) and three fragments 1.0 to 1.2 Mdal in size. The 2.0- to 2.3-Mdal fragment and the two smaller 1.0-Mdal fragments were resistant to further digestion with BamHI (Fig. 6E). By agarose electrophoretic analysis, B2 in K and B3-R in G-K had the same mobilities as the B2 and B3-R fragments in EcoRI-BamHI-digested P8 DNA (Fig. 2). The K fragment was resistant to Hpa I digestion, whereas G-K yielded 3.2- and 2.0-Mdal fragments. The hybridization showed that the uncleaved G-K and K fragments hybridized to the dDNA probe and that all of the G-K and K BamHI and Hpa I cleaved fragments hybridized with the PO DNA probe. In contrast, the G-K 3.0-Mdal BamHI fragment and the 2.0-Mdal Hpa I fragment did not hybridize with the

![Figure 6](http://jvi.asm.org/) Characterization of dDNA sequences related to the BamHI and Hpa I cleavage fragments of the HindIII G-EcoRI K (G-K) and EcoRI K fragments of PO DNA. Purified PO DNA K and G-K fragments (A) were cleaved with BamHI (C) or Hpa I (F) restriction enzymes, separated electrophoretically on agarose gels, stained with ethidium bromide, and by Southern technique (18), hybridized either to 32P-labeled 5.4- to 5.7-Mdal EcoRI dDNA (D and G) or 32P in vitro labeled PO DNA (E and H). G-K (upper arrows in C and F) and its BamHI and Hpa I digests are on the left of each column, whereas the corresponding digests of K (lower arrow in C and F) are to the right. The fragments obtained with Bam HI (3.0 Mdal) and Hpa I (2.0), which hybridized to PO probe but not to dDNA probe, are indicated. B1 and B2 comigrate with B1 of PO and P8 DNA and B3-R of dDNA respectively (Fig. 2), and are obtained with BamHI digestion of EcoRI K. B3-R is the fastest migrating fragment obtained with BamHI digestion of G-K and corresponds in mobility to B3-R of EcoRI-BamHI-digested dDNA (Fig. 2 and B3-R in Fig. 3). B1 of K and B3 of G-K are not found in dDNA, but hybridize with dDNA probe.
dDNA probe. Thus, 3.0 Mdal of the 8.7-Mdal HindIII G fragment was not present in dDNA sequences, whereas the remaining BamHI fragments (~5.7 Mdal) all hybridized to dDNA.

**DISCUSSION**

The Patton strain dDNA fragments obtained after EcoRI digestion are heterogeneous in size with apparent mass ranging from 4.3 to 6.5 Mdal. However, the majority of the molecules clustered between 5.4 and 5.7 Mdal (Fig. 1). The origin of the 4.3- and 6.5-Mdal fragments is unknown. KOS strain dDNA appears to be more heterogeneous with respect to both the number and size of EcoRI fragments obtained and its distribution in CsCl (23). However, some similarity exists between both strains in the cluster of fragments observed between 5 and 6 Mdal. In contrast to the heterogeneity of both the Patton and KOS EcoRI dDNA digests, only two fragments (5.1 and 5.4 Mdal) are obtained from EcoRI digestion of the Justin dDNA. Each fragment appears to be derived from a distinct species of dDNA (4, 5). The heterogeneity differences of EcoRI-digested dDNA derived from the three HSV-1 strains could be a strain-dependent phenomenon; however, it is also possible that other factors could influence heterogeneity (e.g., host cell or MOI).

From these studies, DNA sequences of Patton HSV-1 related to dDNA were identified, and the indicated linear arrangement of the BamHI fragments in both the PO HindIII G DNA fragment and dDNA is given in Fig. 7. These physical maps are based on: (i) the demonstration that BamHI digestion of the 5.4- to 5.7-Mdal EcoRI cluster of fragments yields only B2, B3-R, and a 2.6- to 3.2-Mdal cluster of fragments and (ii) the likely assumption that the linear arrangement of sequences in a single unit of dDNA is the same as that in the related PO DNA sequences. The 3.5-Mdal EcoRI K fragment has been shown to be the S region terminal fragment in HSV-1 DNA (8, 16, 19, 25) and the terminal fragment of Justin dDNA (5). Cleavage of EcoRI K with BamHI (Fig. 6) produced the B1, 2.0- to 2.3-Mdal fragment as well as two 1-Mdal fragments. The increase in amounts of both B1 and EcoRI K in P8 compared with that of PO DNA (Fig. 2A and C) suggests that B1 is terminal in both PO and dDNA. The B1 fragment is also considered to be part of a novel junction formed with the B4 fragment (Fig. 7), giving rise to the 2.6- to 3.2-Mdal cluster of BamHI dDNA fragments (Fig. 2). The fact that B1 strongly hybridizes with the 5.4- to 5.7-Mdal EcoRI dDNA probe (Fig. 6D), but is absent from BamHI digests of the latter, is suggestive evidence for its presence in the novel junction. The variability in the size of the B1 fragment may be similar to the variability observed in the L-region terminal repeat (16) or may be due to incomplete cleavage between B1 and one of the smaller (~0.1 Mdal) BamHI fragments. Only B2 is detected in dDNA, which also suggests that the larger 1-Mdal BamHI fragment of EcoRI K (Fig. 6E) represents incomplete cleavage of B2 and one of the ~0.1-Mdal BamHI fragments. A fragment slightly larger than B3, perhaps corresponding to this incomplete cleavage, has occasionally been detected in P8 DNA (P8, Fig. 2C). At least two 0.1-Mdal BamHI fragments (Fig. 3) must be adjacent to B2 to account for the 1.2-Mdal fragment detected in partial BamHI digests of the EcoRI K fragments (Fig. 6), but their exact position is unknown. Complete BamHI digestion of the G-K fragment yielded 3.0-, 1.2 (B3)- and 1.0 (B3-R)-Mdal fragments, whereas partial digestion gave two additional fragments, 4.4 and 2.4 Mdal, representing incom-

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**Fig. 7.** Diagrammatic presentation of BamHI sites in the HindIII G fragment (G-K and K) and dDNA of HSV-1. (A) Linear arrangement of BamHI (B) sites in the PO HindIII (Hd) G fragment are shown in relation to the published EcoRI (R) and Hpa I (Hp) sites (8, 16, 24, 25). (B) Arrangement of dDNA BamHI sites compared with those of the HindIII G fragment. (- - - -) Indicates the variable region in dHSV probably resulting from the fusion of the terminal B1 fragment and the termination of the dDNA in the B1 fragment. Parentheses indicate possible variable amounts of B1 and B4 fragments appearing in the joint.
complete cleavages of 3.0 Mdal + B4 and B3 + B2-R, respectively. The position of these fragments (Fig. 7) was based on the loss of the B4 site in dDNA, the presence of the EcoRI K site in B3-R, and the lack of dDNA-related sequences in the 3.0-Mdal fragment. The 3.0-Mdal fragment is thus adjacent to the 3.0-Mdal S region HindIII N fragment (16, 24), which also lacks dDNA related sequences (Fig. 4 and 6). Similarly, the 2.0-Mdal Hpa I fragment of G-K did not hybridize with the dDNA probe and was also placed terminal in G-K. This Hpa I site corresponds to the position assigned by Wilke (24) for the generation of S-region terminal 6.9-Mdal Hpa I G fragment.

The 2.6- to 3.2-Mdal cluster of BamHI fragments in dDNA (Fig. 2) would therefore represent the termination of dDNA sequences within the B3 fragment and the recombination of these sequences with all or part of the terminal B1 fragment. The size distribution in the cluster could arise from either a variable termination point in the B1 fragment or variations in the size of B1. Electron microscopy studies of Justin dDNA suggest that the variability is derived from the S region (4, 5), and, as with Justin dDNA (5), the 5.4- to 5.7-Mdal EcoRI component of Patton-defective genomes also originates from the S region.

Virtually all of the Patton dDNA sequences can be accounted for in the related G-K and K sequences. This relationship does not preclude the possibility of other regions giving rise to defective genomes (see Schroeder et al. [13], HSV-1 ANG dDNA lacks EcoRI cleavage sites) but suggests that an origin of replication (4, 5, 16) and a structural predisposition to dDNA formation exists within fragments B3 through B4. It is possible that dDNA is generated in two steps. First a recombination event occurs between sequences in B3 and B4, which gives rise to a replicating circle. Second, linear repeating units are synthesized from the circle with their size selected for by virion packaging requirements. In any case the linear arrangement of these fragments together with their convenient 1- to 3-Mdal size should be useful for identifying the specific sequences associated with the recombination event and dDNA formation.

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LITERATURE CITED


