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Herpesvirus saimiri DNA in Tumor Cells—Deleted Sequences and Sequence Rearrangements

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Herpesvirus saimiri DNA in continuous lymphoblastoid cell lines obtained from viral induced tumors in marmosets has been analyzed by gel electrophoresis of restricted DNA, Southern transfer to nitrocellulose filters, and hybridization to ^{32}P-labeled viral DNA or DNA fragments. The viral DNA fragments *Eco*RI-G, -H, -D, and -I, *Kpn*I-A, and *Bam*HI-D and -E were not detected in Southern transfers of DNA from the nonproducing 1670 cell line. For each restriction endonuclease, a new fragment appeared, consistent with a 13.0-megadalton deletion of viral DNA sequences. This deletion encompassed 35 to 48 ± 0.6 megadaltons from the left end of the unique DNA region. A sequence arrangement map is presented for the major population of *H. saimiri* DNA sequences in the 1670 cell line. Although *H. saimiri* DNA in the nonproducing 70N2 cell line can be distinguished from viral DNA in the 1670 cell line by several criteria, the same sequences were found to be deleted in the major population of viral DNA molecules. Unlike 1670 and 70N2 cells, restricted DNA from the virus-producing cell lines 77/5 and 1926 contained all of the DNA fragments present in the parental virion DNA. DNA from 1670, 70N2, and 77/5 cells contained additional viral DNA fragments that did not comigrate with any virion DNA fragments. Most of these unexplained fragments were confined to or highly enriched in partially purified circular or linear DNA fractions. DNA from tumor cells taken directly from a tumor-bearing animal contained viral DNA indistinguishable from the parental virion DNA by the assay conditions used. These results indicate that viral DNA sequence rearrangements can occur upon cultivation of tumor cells in vitro and that excision of DNA sequences from the viral genome may play a role in establishing the nonproducing state of some tumor cell lines.

Herpesvirus saimiri DNA contains a marked heterogeneity in guanine-cytosine (GC) content that can facilitate its analysis. Unsheared DNA purified from virions is composed of about 90% infectious DNA that bands at a density of 1.705 g/cm^3 (45% G+C) in CsCl and about 10% defective, noninfectious DNA that bands at a density of 1.730 g/cm^3 (71% G+C) in CsCl. The defective DNA consists of identical tandem repeat units of 0.83 megadaltons (Md). Because of its high GC content and density in CsCl this repetitive DNA is called H-DNA. The infectious virion DNA that bands at a density of 1.705 g/cm^3 is composed of 71.6 Md of unique DNA with average GC content of 36% (density in CsCl, 1.695 g/cm^3) to which H-DNA repeat units are covalently attached in the same orientation at each end. Since the number of H-DNA repeat units at each end varies, the size of infectious *H. saimiri* DNA is somewhat variable; the average size of intact infectious DNA is around 100 Md. The infectious virion DNA is called M-DNA, and the central unique 71.6 Md is called L-DNA (1).

H. saimiri is indigenous to the squirrel monkey (*Saimiri sciureus*), in which it causes no apparent disease, but it does produce malignant lymphoma in several other New World primate species (for review, see references 4 and 7). Continuous lymphoblastoid cell lines have been established from *H. saimiri*-induced lymphomas (Table 1), and these may be used to study the properties of the persisting viral DNA in the cultured tumor cells. Previous studies have concentrated on the *H. saimiri* nonproducing tumor cell line 1670. At least a portion of the virus-specific DNA in this cell line is carried as covalently closed circles, similar to those previously found in Epstein-Barr virus-transformed cell lines (14). The circular DNA in 1670 cells, however, is 131 Md, considerably larger than the virion DNA. Examination of partially dena-

tured, circular DNA molecules in the electron microscope revealed two segments of H-DNA (H1 and H2) separating two segments of L-DNA (L1 and L2). Length measurements showed that L1 is 57.8 Md and L2 is 33.7 Md (15). Detailed analysis of 1670 DNA molecules with partially denatured L-DNA regions indicated that L2 sequences were a subset of L1 sequences that represented the leftmost 33.7 Md of virion L-DNA. Furthermore, the common sequences of L2 and L1 were oriented in the same direction. These results thus indicated that approximately 13.8 Md of viral DNA information was absent in circular DNA molecules from 1670 cells (15). Due to the limitations of partial denaturation mapping, the location of the 13.8-Md deletion within L-DNA could not be determined with confidence; the rightmost 24 Md of L1-DNA could not be aligned with virion DNA unambiguously. The results of these partial denaturation analyses of 1670 circular DNA are summarized schematically in Fig. 1.

TABLE 1. *Origin of lymphoblastoid cell lines*

Cell line designation	Strain of virus	Tumor source	Virus-producing or nonproducing[a]	Reference
1670	S295C[b]	Spleen of white-lipped marmoset[c]	Nonproducing	6, 11
70N2	11	Thymus of cotton-topped marmoset[d]	Nonproducing	5
77/5	S295C	Lymph node of cotton-topped marmoset[d]	Producing	9
1926	11	Lymph node of white-lipped marmoset[c]	Producing	5

[a] Determined by cocultivation of approximately 10^6 cells on a 25-cm^2 monolayer of permissive owl monkey kidney cells.

[b] Records indicate that the 1670 cell line was established from a tumor induced with strain S295C, but blot hybridizations of DNA clearly show that presently available 1670 cells contain strain 11 DNA. See the text.

[c] *Saguinus fusicollis-nigricollis.*

[d] *Saguinus oedipus.*

In this report, the Southern transfer procedure (12) has been used to analyze *H. saimiri* DNA sequences in 1670 cells, in three other tumor cell lines, and in tumor cells direct from a tumor-bearing animal. The goals of these experiments were to: (i) confirm the deletion in viral DNA of 1670 cells, (ii) determine the map boundaries of the deletion, (iii) construct a sequence arrangement map for viral DNA of 1670 cells, and (iv) compare the viral DNA of 1670 cells to DNA from other cell lines and cells direct from a tumor-bearing animal.

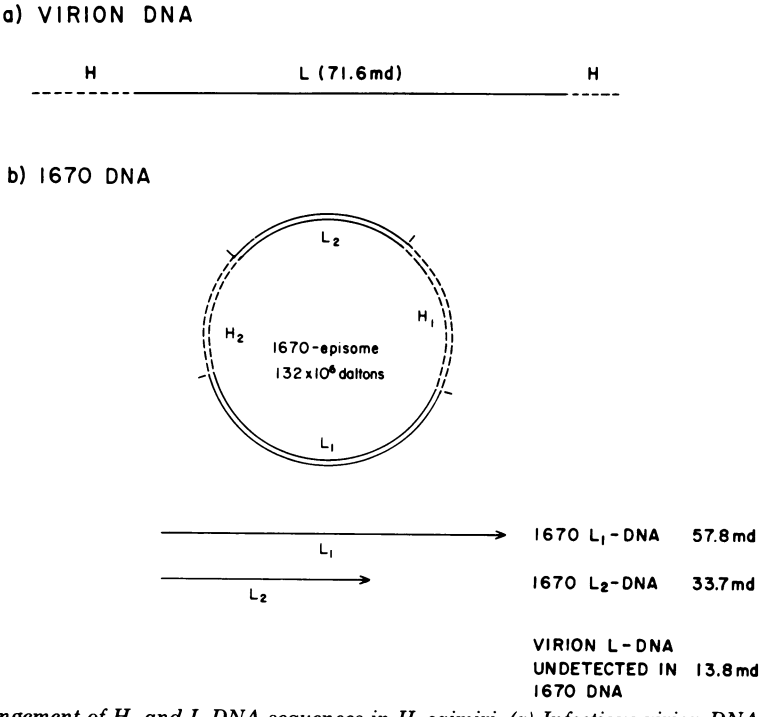

FIG. 1. *Arrangement of H- and L-DNA sequences in H. saimiri. (a) Infectious virion DNA. (b) DNA from 1670 tumor cell line. The structure in (a) is based on Bornkamm et al. (1), and the structure in (b) is from Werner et al. (15).*

MATERIALS AND METHODS

Materials. Materials were obtained from the following sources: cell culture media and serum, GIBCO Laboratories; nitrocellulose filters, Schleicher and Schuell Co.; radioactive supplies, New England Nuclear Corp.; proteinase K, EM Biochemicals; restriction endonucleases, New England Biolabs.

Cells and viruses. *H. saimiri* was grown in permissive owl monkey kidney (OMK line 637) cells and purified from the extracellular supernatant of lysed cells as described (1). The *H. saimiri*-carrying lymphoid cell lines used in this study were initially established from *H. saimiri* experimentally induced tumors in cotton-topped and white-lipped marmoset monkeys (*Saguinus* spp.; Table 1). Cell lines 1670, 70N2, and 77/5 were obtained from B. Fleckenstein in 1978; the cell line 1926 was obtained from a frozen stock of L. Falk in 1979. These cell lines were monitored periodically for virus production by cocultivation of approximately 10^6 cells with a 25-cm^2 monolayer of OMK cells (no higher than passage 30). 1670 and 70N2 cells tested negative, and 77/5 and 1926 tested positive for virus production throughout the course of this study. All lymphoid cell lines were grown as stationary suspension cultures in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, penicillin, and streptomycin. The 1670 and 70N2 cells used in this study have been in continuous culture for over 7 years.

Tumor cell DNA was obtained from peripheral lymphocytes of an owl monkey inoculated with *H. saimiri* strain S295C. DNA was prepared from lymphocytes obtained 275 and 340 days post-inoculation; the animal was sacrificed 342 days post-inoculation. Histopathological examination confirmed a diagnosis of malignant lymphoma. Tumor cells direct from the tumor-bearing owl monkey produced virus when cocultivated with OMK cells.

Stocks of simian virus 40 were grown in Vero cells.

Isolation of DNA. *H. saimiri* M-DNA (density, 1.705 g/cm^3) was prepared from Sarkosyl-lysed virions by equilibrium centrifugation in CsCl (1).

Cellular DNA was also isolated by equilibrium cen-

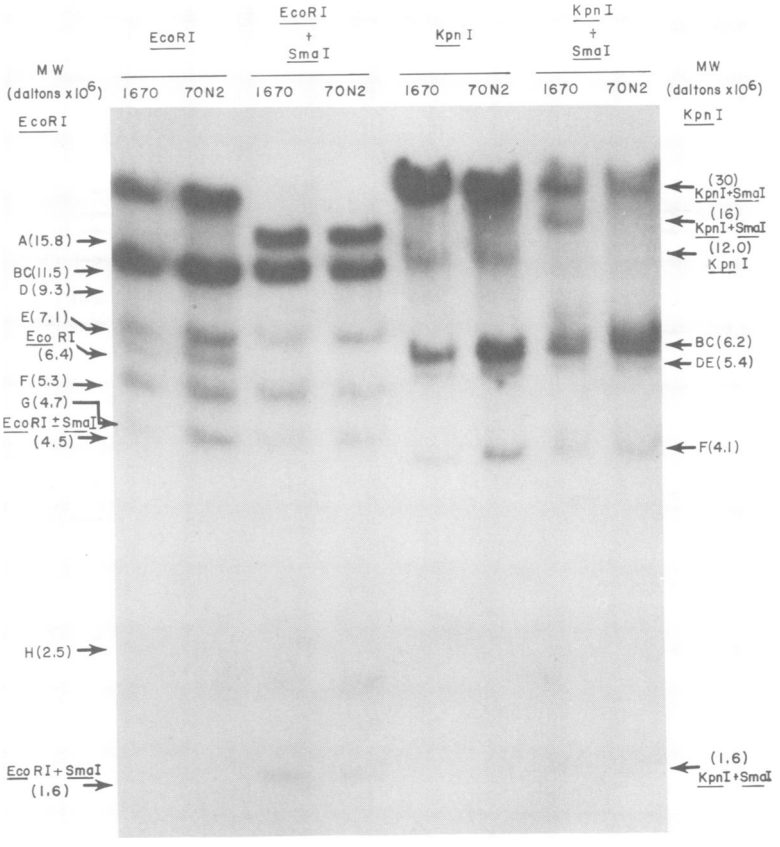

FIG. 2. *Digestion of 1670 and 70N2 total cell DNA by EcoRI with and without SmaI and by KpnI with and without SmaI. Total cell DNA (3 μg) was digested with restriction endonuclease(s), electrophoresed overnight through a 0.5% agarose gel, transferred to a nitrocellulose filter, hybridized to [^{32}P]L-DNA and autoradiographed. The locations of virion DNA fragments are indicated by letters. Fragments different from standard virion DNA fragments are also noted. ±SmaI means that this fragment was observed whether or not SmaI digestion was performed.*

trifugation in CsCl (14). Approximately 1.5×10^8 cells were suspended in 21.2 ml of 75 mM Tris-hydrochloride (pH 9.0)–25 mM EDTA; when linear and superhelical (circular) DNAs were to be prepared in ethidium bromide-cesium chloride gradients, ^3H- or ^{14}C-labeled simian virus 40 DNA was added to the suspended cells at this time. Cells were lysed by the addition of 1.1 ml of 20% Sarkosyl and rocked gently at room temperature for 1 h. Approximately 7.5 mg of proteinase K was added, and protein was digested at 37°C overnight. A 56.4-g amount of CsCl was mixed with 26.4 ml of 50 mM Tris-hydrochloride (pH 9.0), and digested lysed cell solution was added. The refractive index was adjusted to 1.3985 (density, 1.690 g/cm^3), and the solution was centrifuged at 36,000 rpm for 40 h in a 50 Ti rotor of a Beckman ultracentrifuge at 19°C. Fractions were collected from a large hole punctured in the bottom of the tube, and fractions with densities of 1.695 to 1.730 g/cm^3 were pooled. For total cellular DNA, these pooled fractions were dialyzed, and the DNA was precipitated with ethanol and finally suspended in 10 mM Tris-hydrochloride (pH 7.5); after measuring the optical density, EDTA was added to 1 mM. For isolation of linear and superhelical DNA, the pooled fractions were adjusted to a refractive index of 1.387 with 20 mM Tris-hydrochloride (pH 8.0)–1 mM EDTA. Ethidium bromide solution was then added, using subdued light, to a final concentration of 300 μg/ml, and the DNA was centrifuged at 36,000 rpm for 40 h at 19°C, in a 50 Ti rotor. One-milliliter fractions were collected from a large hole punctured at the bottom, and the viscous frac-

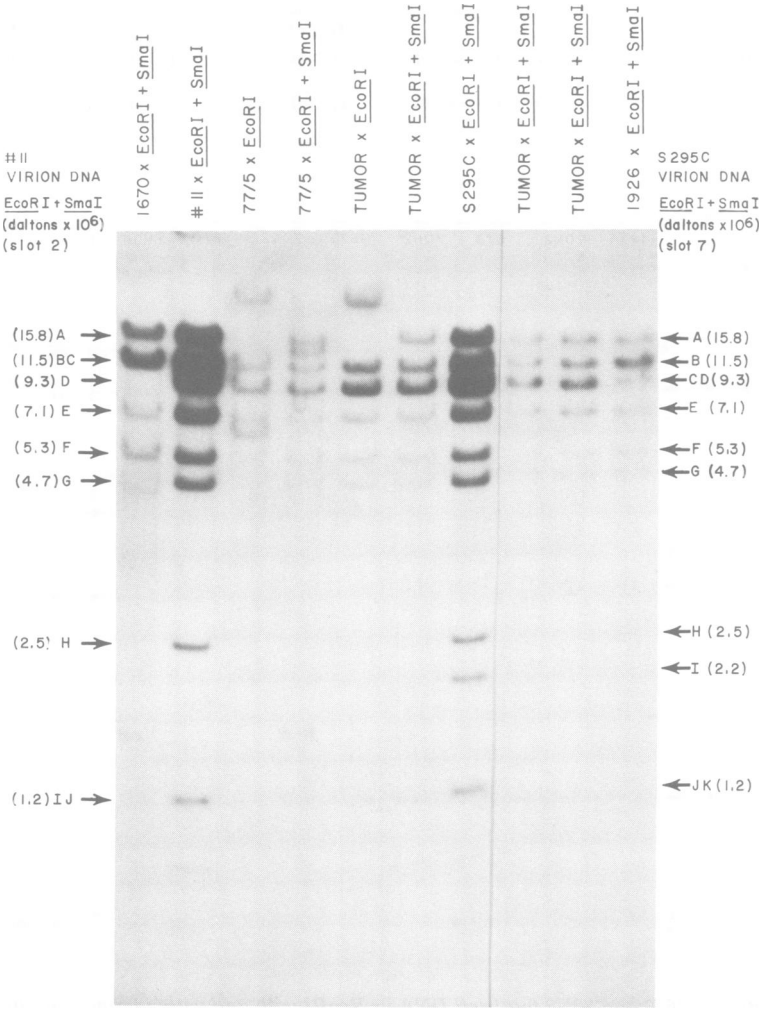

FIG. 3. *Digestion of total DNA from tumor cells and several tumor cell lines by EcoRI with and without SmaI. Total cell DNA (3 μg) or virion DNA (6 ng + 3 μg of OMK DNA) was digested with restriction endonuclease(s), electrophoresed overnight through a 0.5% agarose gel, transferred to a nitrocellulose filter, hybridized to [^{32}P]L-DNA, and autoradiographed. The standard H. saimiri virion DNA fragments are indicated in the left and right margins. Slots 8 through 10 were a different gel than slots 1 through 7.*

tions containing linear cell DNA were noted. Each fraction was analyzed for ^{3}H- or ^{14}C-labeled simian virus 40 DNA and for refractive index. The bulk of cellular DNA viscosity was always well separated from the peak of simian virus 40 superhelical DNA (refractive index, 1.389; density, 1.585 g/cm^3). Fractions containing superhelical DNA and linear DNA were pooled separately, and ethidium bromide was removed by repeated extraction with isoamyl alcohol. Carrier calf thymus DNA (10 to 15 μg) was added to the superhelical (circular) DNA, and preparations were dialyzed versus 10 mM Tris (pH 7.5). Linear DNA was precipitated with ethanol and suspended as described above for total cell DNA. Circular DNA was dialyzed versus water, lyophilized, and suspended in a small volume of 10 mM Tris-hydrochloride (pH 7.5)–1 mM EDTA.

Simian virus 40 DNA was prepared from infected Vero or OMK cells labeled 8 to 72 h postinfection by the procedure of Chou and Martin (2).

Gel electrophoresis and transfer to nitrocellulose filters. DNA samples were digested by restriction endonucleases according to the recommendation of the manufacturer. Digestion was performed for a minimum of 4 and a maximum of 20 h with at least two times more enzyme than necessary to digest the DNA to completion in 15 min. DNA fragments were electrophoresed in 0.5% agarose gels at 50 V (15 mA) as previously described (3). Transfer of DNA fragments from agarose gels to nitrocellulose was performed by the procedure of Southern (12). DNA fragments were usually nicked before denaturation, neutralization, and transfer by soaking the gel (twice for 25 min each) in 0.25 M HCl. The transfer blots shown in Fig. 4–6 and 8 were performed before the nicking procedure was introduced; thus, the transfer of higher-molecular-weight fragments in these figures is probably not in proper proportion to the smaller fragments.

Hybridization of transferred DNA to *H. sai-*

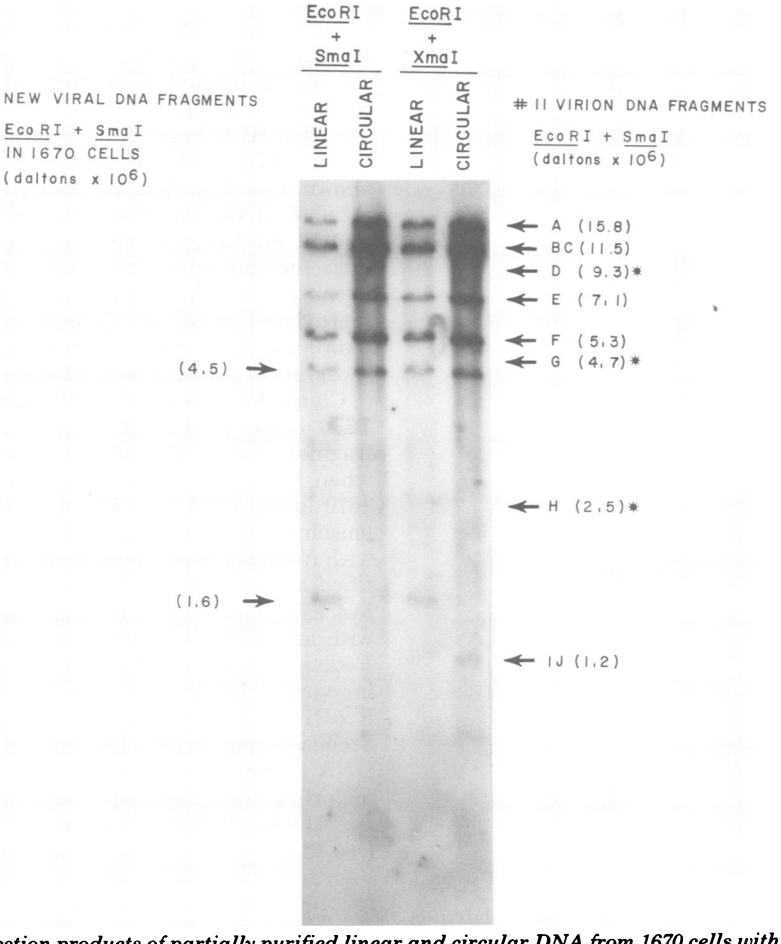

FIG. 4. *Digestion products of partially purified linear and circular DNA from 1670 cells with EcoRI+SmaI and EcoRI+XmaI. Partially purified circular DNA or 3 μg of partially purified linear DNA was digested with restriction endonucleases, electrophoresed overnight through a 0.5% agarose gel, transferred to a nitrocellulose filter, hybridized to [^{32}P]L-DNA, and autoradiographed. *, Indicates virion DNA fragments not detected in viral DNA of 1670 cells.*

***miri* [^{32}P]L-DNA.** *H. saimiri* L-DNA was prepared by sucrose gradient sedimentation of *Sma*I-digested M-DNA. *Sma*I cleaves in the terminal repeat units (H-DNA) but does not cleave in the unique L-DNA (1). *Sma*I-cleaved M-DNA was layered over a 10 to 30% sucrose gradient in 1 M NaCl–0.05 M Tris-hydrochloride (pH 7.5)–1 mM EDTA and centrifuged at 21,500 rpm for 16 h at 20°C in an SW40 rotor. Fractions containing L-DNA (about two-thirds from the top) were located spectrophotometrically and pooled, and the DNA was precipitated with ethanol. L-DNA was labeled with ^{32}P by the "nick repair" method (3, 10). Specific activities of 0.5×10^8 to 1.0×10^8 cpm/μg of DNA (Cerenkov) were usually obtained. Hybridization and rinsing of DNA blot transfers were performed as described previously (3), except that two additional rinsing steps were employed: 1× SSC and 0.2× SSC at 60°C.

Hybridization of transferred DNA to *H. saimiri* ^{32}P-labeled *Eco*RI DNA fragments. For purification of *Eco*RI fragments, 10 μg of *H. saimiri* strain 11 M-DNA was digested with *Eco*RI+*Sma*I and electrophoresed in one wide slot of a 0.5% agarose gel. DNA fragments stained with ethidium bromide were excised and eluted electrophoretically in dialysis bags. Eluted DNA was dialyzed versus water and lyophilized. *H. saimiri Eco*RI DNA fragments were labeled with ^{32}P by the nick repair method as described above and used for hybridization (see legend to Fig. 8).

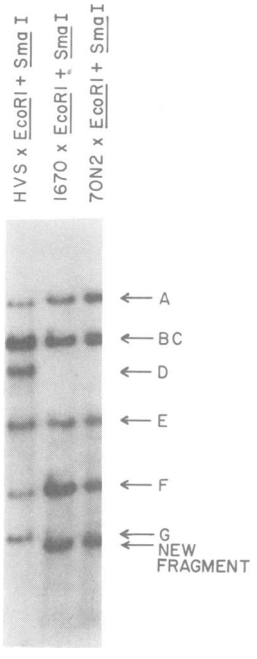

FIG. 5. *Comparison of EcoRI+SmaI digestion products of H. saimiri DNA from virions, 1670 cells, and 70N2 cells. A 3-μg amount of total cell DNA or 6 ng of strain 11 virion DNA was digested with EcoRI+SmaI, electrophoresed for 40 h through a 0.5% agarose gel, transferred to a nitrocellulose filter, hybridized to [^{32}P]L-DNA, and autoradiographed.*

RESULTS

General considerations. Comparative cleavage of virion M-DNA with a restriction endonuclease that cleaves only in L-DNA with and without *Sma*I has been used to locate L-DNA terminal fragments (i.e., at H-L border) since *Sma*I cleaves in H-DNA but not in L-DNA (8). A similar approach has been used here for detecting L-DNA fragments located at the H-L borders in 1670 cells, but in this case the interpretation must consider the highly methylated CG dinucleotides in H-DNA of 1670 cells that inhibit cleavage by *Sma*I (3). *Xma*I has been used instead of *Sma*I in some cases in these experiments since it has the same recognition sequence (CCCGGG) but cleaves whether or not the C of the CG dinucleotide is methylated (H. Youssoufian and C. Mulder, J. Mol. Biol., in press).

H. saimiri DNA fragments in agarose gels were detected by Southern transfer, hybridization to ^{32}P-labeled *H. saimiri* L-DNA or DNA fragments, and autoradiography. *Eco*RI, *Kpn*I, and *Bam*HI linkage maps of virion L-DNA determined by C. Mulder and B. Fleckenstein are shown (see Fig. 10). Although *Xho*I sites in virion L-DNA have been mapped by C. Mulder and B. Fleckenstein, this enzyme did not produce meaningful patterns with 1670 cell DNA because L-DNA in 1670 cells is extensively methylated at CG (R. Desrosiers, unpublished data), and *Xho*I does not cleave when CG of its recognition sequence is methylated (13).

Cleavage products of *H. saimiri* DNA in 1670 cells. (i) *Eco*RI. The *H. saimiri* DNA fragments D, G, H, and I/J were not detected when total 1670 cell DNA (see Fig. 3, slot 1), 1670 linear DNA (see Fig. 4, slot 1), and 1670 episomal DNA (see Fig. 4, slot 2) were digested with *Eco*RI+*Sma*I. *Eco*RI fragment A was identified as being at the H-L border in 1670 cells just as in virion DNA by digestion with *Eco*RI with and without *Sma*I (Fig. 2, slots 1 and 3). *Eco*RI fragment J is located at the H-L border in virion DNA and was not detected in *Eco*RI+*Sma*I digests of 1670 DNA, most likely because of the variable lengths of methylated H-DNA fragments attached to it; a fragment comigrating with *Eco*RI-I/J was detected when *Eco*RI+*Xma*I digestion of 1670 DNA was used (see Fig. 4, slot 4). A new fragment (4.5 Md) of slightly faster mobility than virion DNA *Eco*RI fragment G was found in *Eco*RI and *Eco*RI+*Sma*I digests of 1670 total, linear, and episomal DNA (Fig. 2–4). The slightly faster mobility of the 1670 *Eco*RI 4.5-Md fragment relative to virion *Eco*RI fragment G and the virtual absence of a discrete *Eco*RI-D are best seen in Fig. 5 where the DNA fragments were

run further out into the gel by longer electrophoresis.

In addition to the *Eco*RI fragments described above, *H. saimiri* DNA, unique to or highly enriched in the linear DNA fraction, was also detected. A fragment of 1.6 Md was detected in *Eco*RI+*Sma*I digests of total 1670 cell DNA (Fig. 2, slot 3). This 1.6-Md fragment was highly enriched in the linear DNA fraction with either *Eco*RI+*Sma*I or *Eco*RI+*Xma*I (Fig. 4). An *H. saimiri* DNA fragment of 6.4 Md was detected in *Eco*RI digests of 1670 total cell DNA (Fig. 2, slot 1). This fragment is also enriched in the linear DNA fraction (data not shown).

(ii) *Kpn*I. The *H. saimiri* DNA fragments A and G were not detected when total 1670 cell DNA (Fig. 2, slot 7), 1670 linear DNA (Fig. 6, slot 1), and 1670 episomal DNA (Fig. 6, slot 2) were digested with *Kpn*I+*Sma*I. New fragments of 30 and 16 Md were found in *Kpn*+*Sma*I digests of 1670 total cell DNA (Fig. 2, slot 7) and in *Kpn*I+*Sma*I or *Kpn*I+*Xma*I digests of linear and episomal DNA (Fig. 6). The difference in mobility between the new 30-Md *Kpn*I+*Sma*I fragment in 1670 cells and the virion 43-Md fragment was not obvious in these 0.5% agarose gels run overnight, but electrophoresis for 40 h has clearly shown the difference in mobility and allowed estimation of molecular weight (data not shown). The new 1670 16-Md fragment was identified as being at the H-L border by comparative digestion with *Kpn*I with and without *Sma*I (Fig. 2, slots 5 and 7). *Kpn*I fragment G is located at the H-L border in virion DNA and

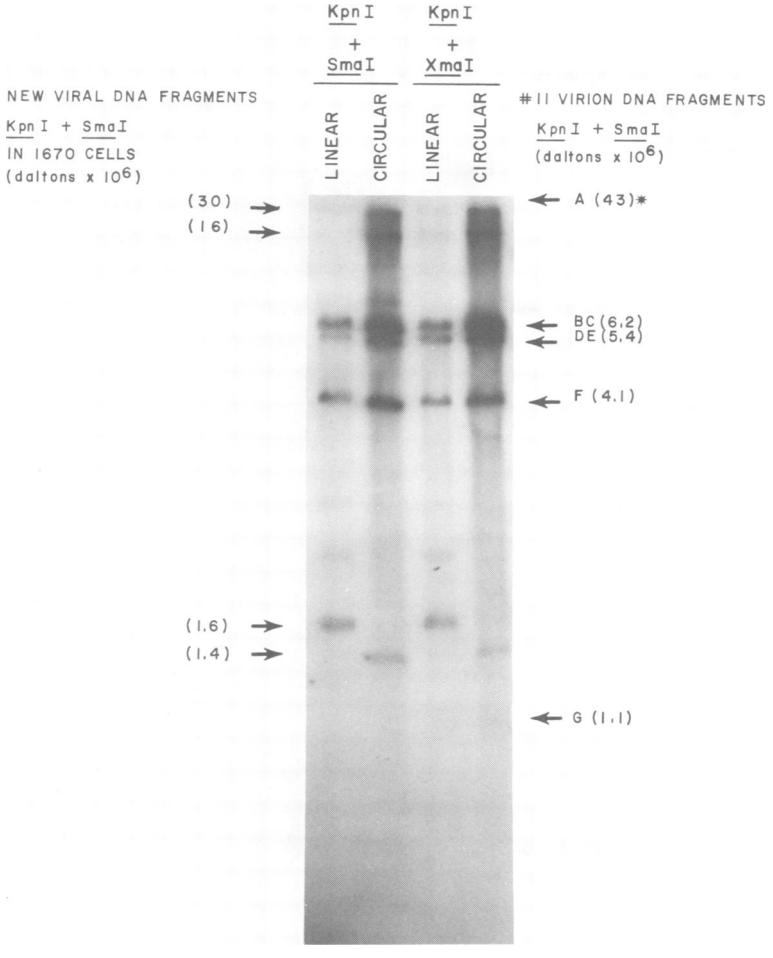

FIG. 6. *Digestion products of partially purified linear and circular DNA from 1670 cells with KpnI+SmaI and KpnI+XmaI. Partially purified circular DNA or 3 μg of partially purified linear DNA was digested with restriction endonucleases, electrophoresed overnight through a 0.5% agarose gel, transferred to a nitrocellulose filter, hybridized to [^{32}P]L-DNA, and autoradiographed. *, Indicates virion DNA fragments not detected in viral DNA of 1670 cells.*

was not detected in *Kpn*I+*Sma*I digests of 1670 DNA, most likely because of methylated H-DNA fragments heterogeneous in size attached to it; a fragment comigrating with *Kpn*I-G was detected when *Kpn*I+*Xma*I digestion of 1670 DNA was used (Fig. 6, slot 4). The putative assignment of one of the *Kpn* D/E fragments to the H-L border is more difficult since one must depend on a difference in intensity due to the comigration of the fragments and since heterogeneously sized methylated H-DNA fragments probably affect the mobility of a fragment even as large as 5.4 Md. The results, however, shown in Fig. 6, are consistent with one of the fragments being at the H-L border.

In addition to the *Kpn*I fragments described above, *H. saimiri* DNA in 1670 cells, unique to or highly enriched in the linear DNA fraction and the circular DNA fraction, was also detected. A fragment of 1.6 Md was found to be highly enriched in *Kpn*+*Sma*I or *Kpn*I+*Xma*I digests of 1670 linear DNA (Fig. 6, slots 1 and 3). A fragment of 1.4 Md was found to be highly enriched in *Kpn*I+*Sma*I or *Kpn*I+*Xma*I digests of 1670 episomal DNA (Fig. 6, slots 2 and 4). When 1670 total cell DNA was digested with

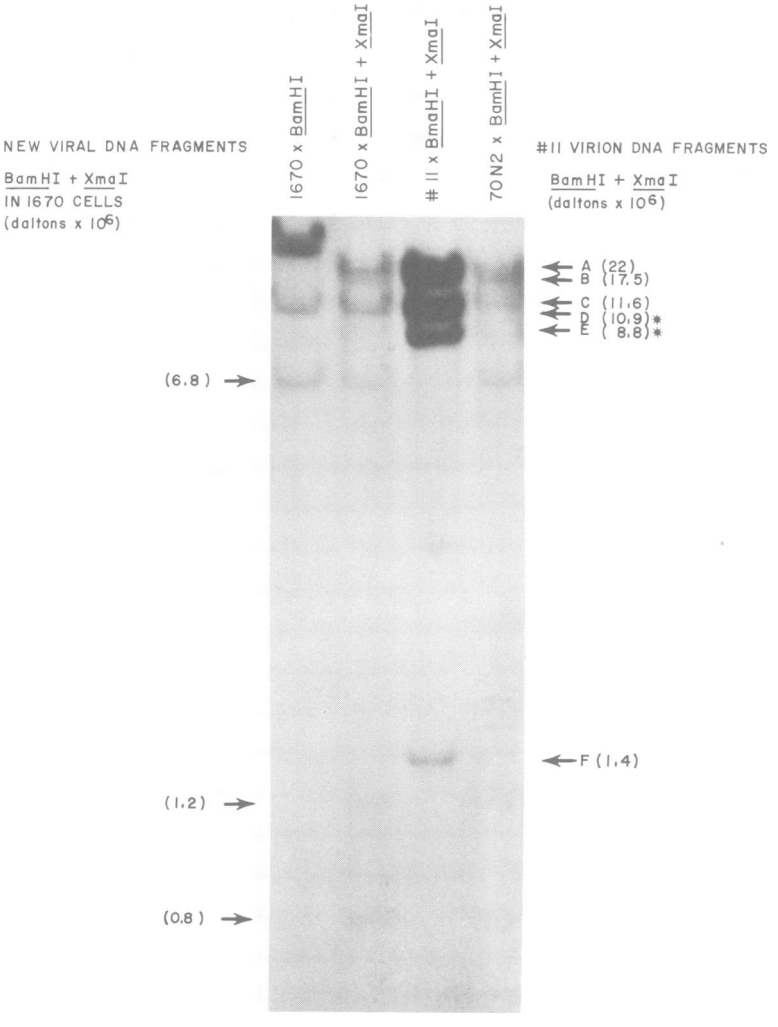

FIG. 7. *Digestion of total DNA from 1670 and 70N2 cells by BamHI with and without XmaI. Total cell DNA or virion DNA was digested with restriction endonuclease(s), electrophoresed overnight through a 0.5% agarose gel, transferred to a nitrocellulose filter, hybridized to [^{32}P]L-DNA, and autoradiographed. Fragments different from any virion DNA fragment are indicated in the left margin. *, Indicates virion DNA fragments not detected in viral DNA of 1670 and 70N2 cells.*

*Kpn*I+*Sma*I, the 1.6-Md fragment was two to three times more intense than the 1.4-Md fragment (data not shown).

An *H. saimiri* fragment of 12.0 Md was detected in *Kpn*I digests of 1670 total cell DNA (Fig. 2, slot 5). It is not known whether this fragment is highly enriched in either the episomal or linear DNA fractions.

(iii) *Bam*HI. *H. saimiri* fragments D and E were not detected when total 1670 cell DNA was digested with *Bam*HI alone or with *Bam*HI+*Xma*I. A new fragment in 1670 cells of 6.8 Md was present after cleavage with *Bam*HI alone or with *Bam*HI+*Xma*I (Fig. 7, slots 1 and 2). *Bam*HI fragments A and B were identified as being at the H-L border in 1670 cells by comparative digestion with *Bam*HI with and without *Xma*I (Fig. 7, slots 1 and 2). In addition, new fragments of 1.2 and 0.8 Md were found in *Bam*HI+*Xma*I digests of 1670 DNA (Fig. 7, slot 2).

(iv) Hybridization to ^{32}P-labeled virion DNA fragments. *Eco*RI fragments were prepared as described above and labeled with ^{32}P by the nick repair method. The individual ^{32}P-labeled fragments were used as hybridization probes to Southern blots of total 1670 cell DNA cleaved with *Eco*RI+*Sma*I and total 1670 cell DNA cleaved with *Bam*HI+*Sma*I. The purpose of this experiment was twofold: (i) to see whether any hybridization could be detected with ^{32}P-labeled D and H probes, and (ii) to determine which *Eco*RI fragments hybridize to the new 1670 *Eco*RI fragment of 4.5 Md and the *Bam*HI fragment of 6.8 Md. This could provide evidence as to whether these new fragments arise because of a deletion within the contiguous *Eco*RI fragments G, H, D, and I, and *Bam*HI fragments D and E.

^{32}P-labeled *Eco*RI virion DNA fragments B/C and E hybridized strongly with their comigrating fragments in 1670 DNA (Fig. 8, slots B/C and E). ^{32}P-labeled *Eco*RI virion DNA fragments B/C and E also gave considerable additional hybridization, especially to DNA in the 2- to 4-Md range. ^{32}P-labeled *Eco*RI virion DNA fragments D and H did not hybridize significantly with any comigrating fragments in 1670 DNA but possibly did hybridize to heterogeneously migrating material, especially in the 2- to 4-Md range (Fig. 8, slots D and H). ^{32}P-labeled *Eco*RI virion DNA fragment G hybridized primarily to the new 1670 *Eco*RI fragment of 4.5 Md (Fig. 8, slot G). *Eco*RI fragments G and E but not B/C, D, or H hybridized to the new 1670 *Bam*HI fragment of 6.8 Md (data not shown). It is not possible to tell what potential impurities in the purified fragments contributed to the observed hybridization.

***H. saimiri* DNA deletion in 70N2 cells compared with the deletion in 1670 cells.** Comparison of *H. saimiri* DNA in total 1670 and 70N2 cell DNA digested with *Eco*RI with and without *Sma*I and *Kpn*I with and without *Sma*I is shown in Fig. 2. Digestion with *Eco*RI, *Eco*RI+*Sma*I, and *Kpn*I could not distinguish 1670 and 70N2 DNA. The 16-Md *H. saimiri* DNA fragment in *Kpn*I+*Sma*I digests of 1670 DNA, however, was not detected in *Kpn*I+*Sma*I digests of 70N2 DNA. The new *Eco*RI 4.5-Md fragment was identical in size (as well as can be determined by agarose gel electrophoresis) in 1670 and 70N2 cells (Fig. 5). The similarity of *H. saimiri* DNA fragments from 1670 and 70N2 cells suggested experiments to eliminate the possibility of cross-contamination of the cell lines

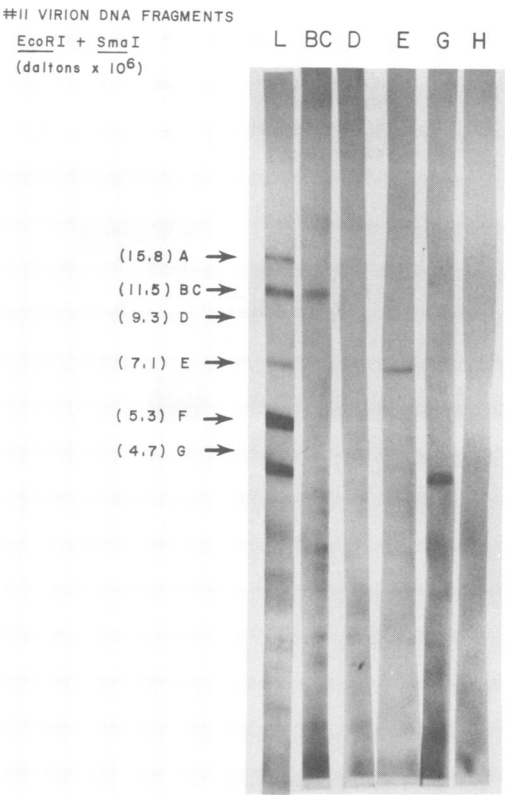

FIG. 8. *Hybridization of purified H. saimiri ^{32}P-labeled EcoRI fragments to Southern blots of 1670 DNA digested with EcoRI+SmaI after agarose gel electrophoresis. Total 1670 DNA was digested with EcoRI+SmaI, electrophoresed in one wide slot through a 0.5% agarose gel, and transferred to a nitrocellulose filter. Strips from this Southern blot were used for hybridization to [^{32}P]L-DNA (lane L) or ^{32}P-labeled EcoRI fragments. The ^{32}P-labeled EcoRI fragment used for hybridization is indicated at the top of each lane.*

during these experiments. 1670 and 70N2 cells independently obtained and maintained for several years were kindly sent by R. Neubauer in Frederick, Md., and digests of these DNAs behaved identically to the 1670 and 70N2 DNA digests shown in Fig. 2 (data not shown). DNA from the 1670 and 70N2 cells sent by R. Neubauer also showed the same H-DNA methylation difference previously observed in 1670 and 70N2 cells maintained in our laboratory (3). DNA isolated at the same time that the original partial denaturation studies were done (14, 15) was sent by B. Fleckenstein, and cleavage patterns of this DNA were indistinguishable from the cleavage patterns of DNA described here (data not shown).

***H. saimiri* DNA in the producing lines 77/5 and 1926.** Digestion of 77/5 total cell DNA with *Eco*RI+*Sma*I and *Kpn*I+*Sma*I produced all fragments seen with equivalent digestions of *H. saimiri* strain S295C virion DNA (Fig. 3 and 9). Similarly, digestion of 1926 total cell DNA with *Eco*RI+*Sma*I produced the same fragments seen with *H. saimiri* strain no. 11 virion DNA (Fig. 3, slots 2 and 10; the lower-molecular-weight fragments were evident on longer film exposures). One of the *Eco*RI B/C fragments of strain no. 11 virion DNA has an additional

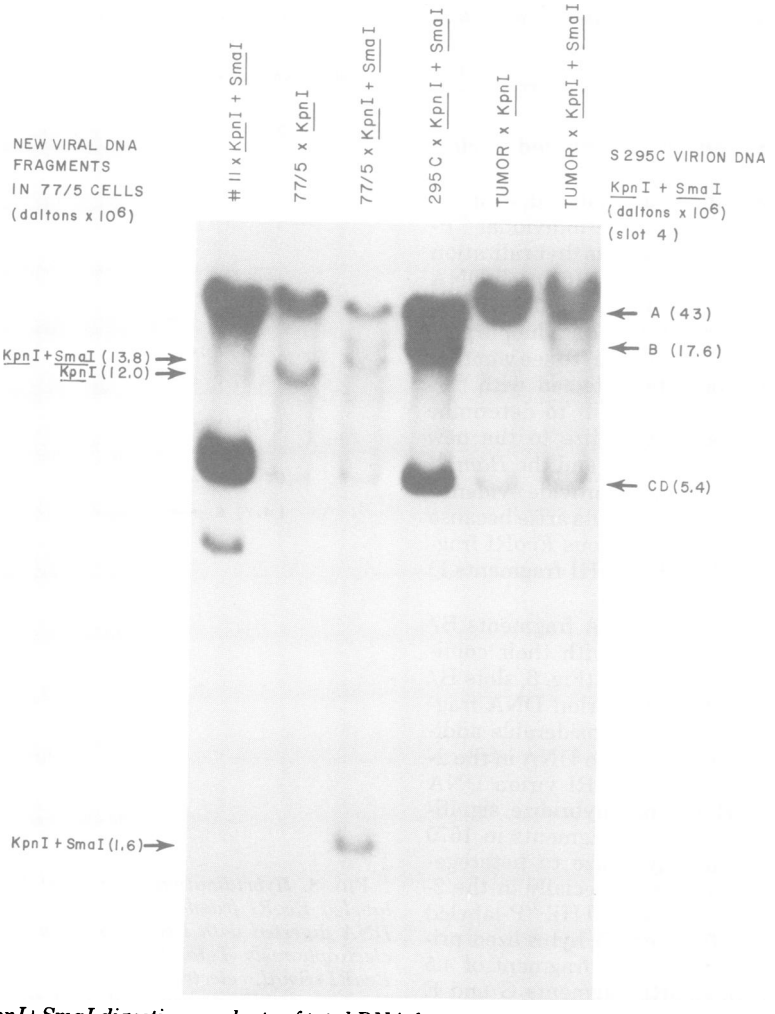

FIG. 9. *KpnI+SmaI digestion products of total DNA from tumor cells and from the 77/5 cell line. Total cell DNA (3 μg) or virion DNA (6 ng + 3 μg of OMK DNA) was digested with restriction endonuclease(s), electrophoresed overnight through a 0.5% agarose gel, transferred to a nitrocellulose filter, hybridized to [^{32}P]L-DNA, and autoradiographed. 77/5 DNA fragments different from any virion DNA fragments are indicated in the left margin.*

*Eco*RI cleavage site in strain S295C, producing additional fragments of 9.3 and 2.2 Md. The *Kpn*I cleavage sites in strain no. 11 DNA, producing fragments G, B, C, and F, are absent in strain S295C, producing one much larger fragment of 17.5 Md. No additional discrete *H. saimiri* DNA fragments were seen in the *Eco*RI+*Sma*I digest of 1926 cell DNA, but the following additional *H. saimiri* DNA fragments were found in 77/5 DNA digests: *Eco*RI, 6.4 Md; *Eco*RI+*Sma*I, 13.2 and 1.6 Md; *Kpn*I, 12.0 Md; and *Kpn*I+*Sma*I, 13.8 and 1.6 Md (Fig. 3, slots 3 and 4 and Fig. 9, slots 2 and 3). Virion DNA produced by cocultivation of 77/5 cells with permissive OMK cells did not contain these unusual fragments (data not shown).

***H. saimiri* DNA in tumor cells of a tumor-bearing animal.** *Kpn*I, *Kpn*I+*Sma*I, *Eco*RI, and *Eco*RI+*Sma*I digestion of DNA from tumor cells of a tumor-bearing owl monkey produced viral DNA fragments identical to the strain of virus used for inducing the tumor (S295C) (Fig. 3, slots 5 and 6 and Fig. 9, slots 5 and 6). No additional *H. saimiri* DNA fragments were detected.

DISCUSSION

The restriction endonuclease cleavage pattern of DNA from each strain of *H. saimiri* is distinctive. Records on the establishment of the 1670 cell line (11 and L. Falk, personal communication) indicate that this cell line was established from a tumor induced with strain S295C. It is obvious from the DNA cleavage patterns in this report and others not shown that the 1670 cell line contains strain no. 11 *H. saimiri* DNA. The present 1670 cell line may have arisen by a cell cross-contamination that occurred early in its passage history. All other *H. saimiri* cell lines grown in this laboratory (70N2, 77/5, 1926, H1591, and MLC-1) can be distinguished from the 1670 cell line and from each other by their ability to produce virus and by Southern blot hybridization analysis (R. Desrosiers, unpublished data).

The hybridization data presented here are consistent with previous partial denaturation results and are sufficiently complete to allow presentation of the model of *H. saimiri* DNA sequence arrangement in 1670 cells shown schematically in Fig. 10. In addition to the partial denaturation results, the following can be cited as evidence for this structure. (i) The absence of *Kpn*I fragment A, *Eco*RI fragments G, H, D, and I, and *Bam*HI fragments D and E in digests of 1670 cell DNA. (ii) The appearance of the following new fragments: *Kpn*I, 30 Md; *Kpn*I+*Sma*I, 16 Md; *Eco*RI, 4.5 Md; and *Bam*HI, 6.8 Md. (iii) The *Kpn*I 30-, *Eco*RI 4.5-, and *Bam*HI 6.8-Md fragments are not further cleaved by *Sma*I or *Xma*I and thus are internal L-fragments. (iv) Isolated *Eco*RI fragment G hybridizes to 1670 DNA new fragments of *Eco*RI (4.5 Md) and *Bam*HI (6.8 Md). The difference in size between *Eco*RI fragments G, H, D, and I and the new 1670 *Eco*RI 4.5-Md fragment is 13.1 Md. The difference in size between *Bam*HI fragments D and E and the new 1670 *Bam*HI 6.8-Md fragment is 12.9 Md. The difference in size between *Kpn*I fragment A and the new 1670 *Kpn* 30-Md fragment is 13.0 Md; estimation of the size of the deletion using *Kpn*I fragments is the least accurate because of the size of the fragments being measured. The size of the deletion calculated here agrees well with the size determined by difference measurements with the electron microscope, $71.6 - 57.8 = 13.8$ Md (15). The results described here unambiguously place the deletion in L1 between 35 and 48 ± 0.6 Md from the left end of L-DNA. The orientation of L1 and L2 in the same direction is derived from previously published partial denaturation mapping (15).

The sequence arrangement of *H. saimiri* DNA in 1670 cells shown in Fig. 10 represents the major population of *H. saimiri* DNA, but it must be stressed that there are still deficiencies in our understanding of viral DNA in 1670 cells. For example, it is not known whether any *H. saimiri* DNA sequences are integrated with host cell sequences. Also, the nature of the new, discrete fragments that do not comigrate with any virion DNA fragments and that are highly enriched in partially purified linear or circular DNA fractions is not known. These new discrete unexplained fragments could be part of other sequence arrangements, such as highly defective *H. saimiri* DNA molecules or sequences integrated within host cell reiterated sequences. Since the size of many of these unexplained fragments is the same in three of the four cell lines examined (1670, 70N2, and 77/5), it seems likely that they have arisen through some common mechanism. The structure of *H. saimiri* DNA in 1670 cells shown in Fig. 10 may not include finer sequence arrangement changes that would not have been detected with the restriction endonucleases used. Such changes could include small deletions or insertions of L-DNA sequences into H-DNA.

The similarity of restriction fragments in 1670 and 70N2 cells indicates that viral DNA sequences deleted within *Eco*RI fragments G, H, D, and I in 1670 cells are deleted identically or nearly identically in 70N2 cells. Viral DNAs from these two cell lines, however, are not identical in other ways, and it is not a simple case of

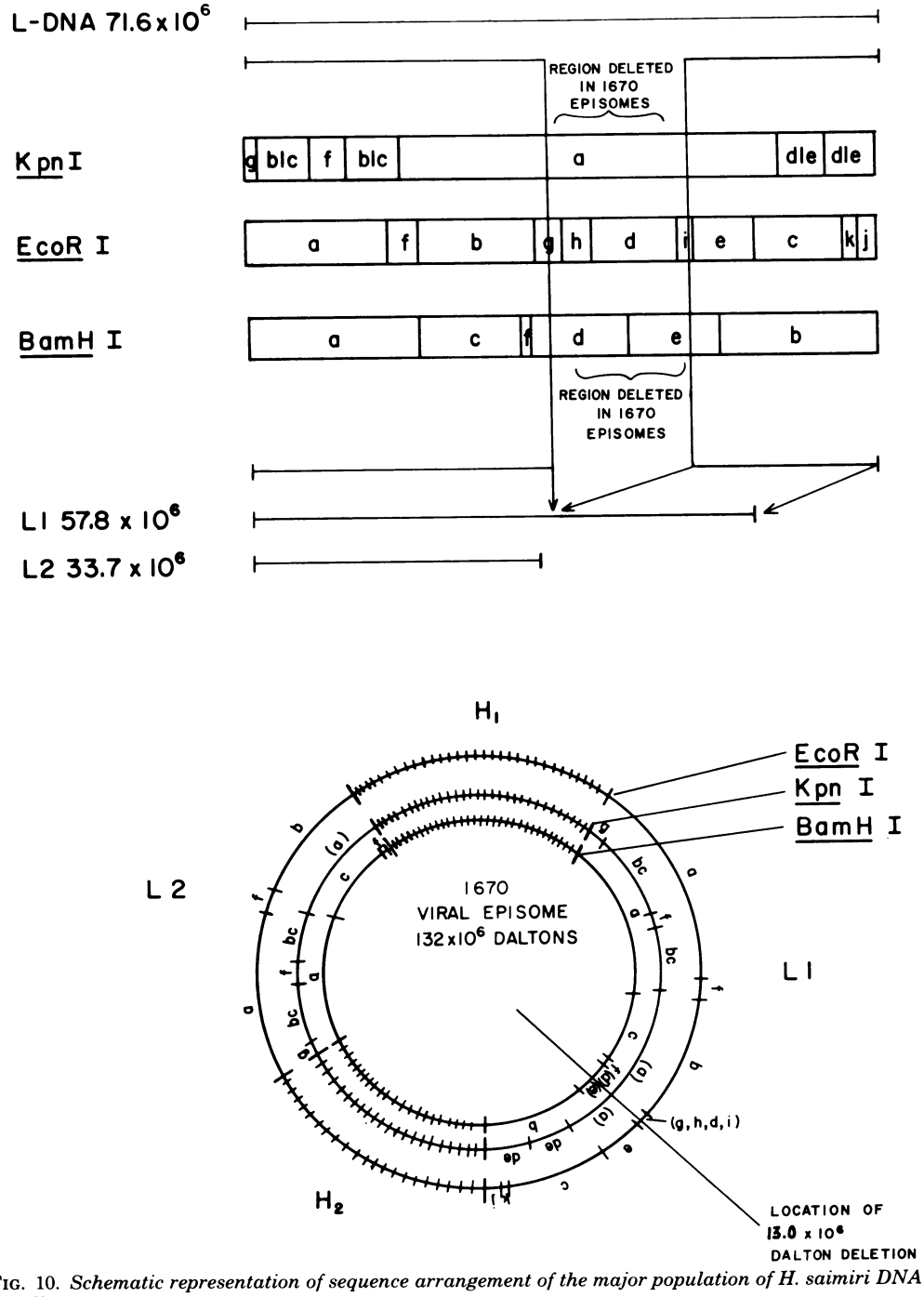

FIG. 10. *Schematic representation of sequence arrangement of the major population of H. saimiri DNA in 1670 cells.*

these being the same cell line. Previously, viral circular DNA was found to be larger in 1670 cells than in 70N2 cells (131 versus 110 Md) (15). Also, viral H-DNA was found to be more extensively methylated in 1670 cells than in 70N2 cells (3), and this has been repeated with the DNAs used in the studies described here and with 1670 and 70N2 cells obtained elsewhere. The absence of the *Kpn*I+*Sma*I fragment of 16 Md in 70N2 cells suggests that the L2 region is absent in

70N2 cells or different from the L2 region in 1670 cells. Consistent with the blot hybridization data, C. Kaschka-Dierich has recently found through partial denaturation mapping that a circular DNA molecule from 70N2 cells contains an L1 region the same size as L1 in 1670 cells and an L2 region of approximately 18 Md (personal communication). Thus, surprisingly similar deletions and rearrangements appear to have occurred independently in the 1670 and 70N2 cell lines.

In addition to the discrete fragments, considerable heterogeneous material was also detected. For example, *Eco*RI+*Sma*I digestion of 1670 DNA produced hybridizing sequences migrating heterogeneously primarily in the 1.6- to 4.5-Md range (Fig. 2, slot 3; Fig. 3, slot 1; and Fig. 4). It is difficult to be certain that the heterogeneously migrating hybridizing sequences are real and not some sort of artifact. The heterogeneously migrating hybridizing sequences were specifically found in continuous tumor cell lines; 6 ng of *H. saimiri* virion DNA plus 3 μg of OMK DNA cleaved with *Eco*RI+*Sma*I did not produce detectable levels of these heterogeneously migrating sequences nor did DNA from tumor cells direct from a tumor-bearing animal (Fig. 3). Their presence did not correlate with the age of the DNA preparation, and these sequences were found in the three 1670 DNA preparations examined. Furthermore, the heterogeneously migrating hybridizing sequences were also detected when ^{32}P-labeled purified fragments were used for hybridization (Fig. 8). The heterogeneously migrating hybridizing sequences could conceivably be part of sequence arrangements such as defective DNA molecules or viral DNA sequences integrated within host cell unique sequences.

Definitive demonstration of the integration of viral DNA within host sequences in growth-transformed cells has been difficult to achieve with the herpesviruses. In this report it has been shown that there are viral DNA sequences highly enriched in a partially purified linear DNA fraction. Whether DNA sequences unique to the linear DNA fraction or DNA in the heterogeneously migrating sequences contain covalently joined host and viral DNA sequences must await further investigation.

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