Herpesvirus ateles DNA and Its Homology with *Herpesvirus saimiri* Nucleic Acid

BERNHARD FLECKENSTEIN,^{1,2*} GEORG W. BORNKAMM,² CAREL MULDER,³ FRED-JOCHEN WERNER,² MUTHIAH D. DANIEL,¹ LAWRENCE A. FALK,⁴ AND HAJO DELIUS⁵

Institut für Klinische Virologie der Universität Erlangen-Nürnberg, 8520 Erlangen, Germany²; New England Regional Primate Research Center, Harvard Medical School, Southboro, Massachusetts 01772¹; Departments of Pharmacology and Microbiology, University of Massachusetts Medical School, Worcester, Massachusetts 01605³; Department of Microbiology, Rush Presbyterian-St. Luke's Medical Center, Chicago, Illinois 60612⁴; and EMBO Research Institute, 6900 Heidelberg, Germany⁵

Received for publication 1 July 1977

Analysis of the structural organization of *Herpesvirus ateles* DNA shows that two types of viral DNA molecules are encapsidated in virions: (i) M-genomes, which contain 74% light sequences (L-DNA, 38% guanine plus cytosine) and 26% highly repetitive heavy sequences (H-DNA, 75% guanine plus cytosine), and (ii) defective H-genomes, which consist exclusively of repetitive H-DNA. The structure of M-genomes from *H. ateles* consists of an L-DNA region of about 70×10^6 daltons inserted between H-DNA termini of variable length. M-genomes with a shorter H-DNA region at one end of the molecule have a long stretch of H-DNA at the other end, resulting in a total molecular weight of $89.8 \pm 8.5 \times 10^6$. Thus it resembles the structure of M-genomes of *H. saimiri*. H-DNA of the two independent *H. ateles* isolates, strains 810 and 73, reveals different patterns after cleavage with restriction endonuclease *Sma* I. H-DNA of *H. ateles* 810 appears to consist of identical tandem repeat units with a molecular weight of 1,035,000; the H-DNA repeat unit of strain 73 is shorter (930,000 molecular weight). Corresponding DNA sequences of the two *H. ateles* strains (810 and 73) are completely homologous in cross-hybridizations. However, a discrete nucleotide sequence divergence between these virus strains is detected by measuring melting temperatures (T_m) of DNA hybrid molecules. Some homology exists between *H. ateles* and *H. saimiri* DNA. Hybridization of L-DNA from *H. ateles* with L-DNA from *H. saimiri* shows about a 35% homology between the respective L-DNA sequences; the resulting heteroduplex molecules show a decrease of T_m by 13.5°C, corresponding to about a 9% mismatching in cross-hybridizing parts of L-regions. Very little homology is found between H-DNA of *H. ateles* and *H. saimiri*.

Herpesvirus ateles is ubiquitous in spider monkeys (*Ateles* sp.) that are infected without overt disease. The virus (strain 810) was isolated originally from kidney cell cultures of species *Ateles geoffroyi* and has been identified as a new member of the herpesvirus group by Meléndez and co-workers (24). Additional *H. ateles* isolates, including strain 73, have been obtained from leukocytes of another spider monkey species (*Ateles paniscus*) (4).

H. ateles is highly oncogenic in various New World primates (16, 24). Experimental infection of these animals is followed by rapid neoplastic proliferation of lymphatic cells within a few weeks or months after inoculation.

Thus, *H. ateles* resembles *Herpesvirus saimiri*, which is a well-characterized virus of squirrel monkeys (5, 6, 17-19, 22, 23) with a high

potential to induce malignant tumors of the lymphatic system in primates. However, the viruses differ in some distinct properties: (i) *H. ateles* and *H. saimiri* have different surface antigens and are therefore distinguished by neutralization in cell culture (24). (ii) *H. ateles* induces fusion of permissive tissue culture cells forming characteristic polycaryocytes, which are not found in *H. saimiri*-infected cells. (iii) *H. ateles* is capable of transforming lymphatic primate cells in vitro (7), whereas *H. saimiri* is not. (iv) Both viruses differ in the host range for induction of malignant lymphomas (20).

In *H. saimiri* virions, two types of DNA molecules are found, M-genomes and H-genomes (8). The M-genome molecule of *H. saimiri* consists of 72% unique light sequences (L-DNA) (36% guanine plus cytosine [G+C]) and 28%

highly repetitive heavy sequences (H-DNA) (71% G+C) (9, 13). L-DNA sequences with a constant length corresponding to 71.6×10^6 molecular weight form the middle part of the M-genome, whereas both ends are stretches of H-DNA of variable length. The H-DNA sequences consist of identical repeat units (molecular weight, 830,000) in tandem orientation (1). M-DNA of *H. saimiri* is infectious in permissive cell cultures (9), and inoculation of purified M-DNA into susceptible monkeys results in tumor growth (10, 12).

The H-genome of *H. saimiri* consists of long molecules of highly repetitive DNA. Sequences of H-genomes are identical to terminal H-sequences in M-genomes. A lack of infectivity of H-genomes corresponds to the low genetic complexity (1, 8).

In the present study the structure of *H. ateles* DNA is investigated and compared with that of *H. saimiri*. The relationship of these two viruses is described based on analysis of nucleotide sequence homologies of their genomes.

MATERIALS AND METHODS

Viruses and cell culture. *H. ateles* 810 and 73 were propagated on OMK cells, an established line of owl monkey kidney cells, growing in monolayer cultures in minimum essential medium with 10% heat-inactivated fetal calf serum. Infected cells were replenished with fresh medium twice weekly until cytopathic changes became visible. For some experiments, infected cells were subcultured by mixing them with uninfected cells.

H. ateles 73 is permanently shed by the *H. ateles*-transformed lymphoid cell line 22CM37 (7) upon cocultivation with OMK cells. The 22CM37 line was cultured in RPMI 1640 medium with 10% heat-inactivated fetal calf serum and 80 µg of gentamycin per ml. Suspension cultures were kept in closed Erlenmeyer flasks and subcultured at 4-day intervals.

H. saimiri 11 was grown on OMK cells as described previously (13).

Herpes simplex virus type 2 strain Kluger was a fresh isolate from a genital lesion. It was typed by DNA-cRNA hybridization (28). The virus was passaged five times at low input multiplicity (<0.001 PFU/cell) before purification of viral DNA.

Preparation of viral and cellular DNA. Viral DNA was labeled by adding 5 µCi of [³H]thymidine (specific activity, 15 to 25 Ci/mmol, Amersham-Buchler, Braunschweig, Germany) per ml to infected cultures about 10 days before harvesting.

H. ateles was partially purified from cytoplasm of infected cells and from culture fluids as described for *H. saimiri* (1). Concentrated virus particles were disintegrated by 2% (wt/vol) sodium lauryl sarkosinate at 60°C for 60 min, and the DNA was centrifuged to equilibrium in CsCl at 30,000 rpm for 72 h in a Spinco 50 Ti rotor. Fractions containing M-DNA or H-DNA, respectively, were pooled and dialyzed against 20 mM Tris-hydrochloride (pH 8.5) and 0.1 mM EDTA.

DNA from tissue culture cells was isolated by den-

sity centrifugation in KI gradients (34).

Analytical ultracentrifugation. Determination of buoyant densities of viral DNA in CsCl was carried out essentially as described previously (9). Partially purified concentrated virus was lysed with 0.4% sodium lauryl sarkosinate in TNE buffer (10 mM Tris-hydrochloride [pH 8.5], 0.1 M NaCl, and 1 mM EDTA) at 60°C for 60 min. CsCl (500 mg) was dissolved in 400 µl of the lysate; to avoid shear-induced breakage, the solution was entered slowly into the centrifuge cells of a Spinco Ti ANF 4 place rotor. DNA was spun to equilibrium in a model E centrifuge at 34,000 or 44,000 rpm for 24 h at 25°C and scanned at a 262-nm wave length.

Partial denaturation mapping of *H. ateles* DNA. DNA in 0.2 M sodium acetate, 20 mM Tris-hydrochloride (pH 8.5), and 0.1 mM EDTA was precipitated by 2.5 volumes of ethanol at -20°C for 30 min and collected in a Spinco SW56 polyallomer tube at 35,000 rpm and 0°C for 30 min. Concentrated DNA was partially denatured by 7 M sodium perchlorate and 1.04 M formaldehyde at low temperature and neutral pH as described in detail elsewhere (1, 32); subsequently, the DNA molecules were passed through a Sephadex G100 column and spread by the formamide technique (3).

DNA reassociation kinetics and determination of melting temperatures of DNA hybrids. Lyophilized DNA was dissolved in 300 µl of water, 50 µl of 2 M NaOH, and 50 µl of NES (3M NaCl, 0.1 M EDTA, and 0.5% sodium lauryl sarkosinate). The DNA was denatured and fragmented by heating at 100°C for 10 min. The solution was neutralized by adding 50 µl of 2 M HCl and 50 µl of 2 M Tris-hydrochloride, pH 7.5. DNA was incubated at 67°C to allow reassociation. This temperature is within an optimal range for both H- and L-DNA (11).

Separation of single-stranded and duplex DNA by hydroxyapatite chromatography, counting of DNA fractions, and determination of the reassociation in a second-order-rate plot (33) were done as reported previously (9).

Melting of L-DNA was performed in 0.14 or 0.4 M sodium phosphate (pH 6.8) by incubating successively for 2 min at each temperature (2°C increments). A portion was removed at each temperature, diluted to 0.14 M sodium phosphate (pH 6.8), and chromatographed on hydroxyapatite at 60°C. Analogous melting of H-DNA hybrids was done in 0.14 M sodium phosphate, pH 6.8.

Cleavage by endonuclease R·Sma I and gel electrophoresis. Viral DNA was digested with restriction endonuclease *Sma* I and analyzed by electrophoresis in 5% acrylamide gels as described previously (1). To quantitate the relative amount of radioactivity in endo R·Sma I fragments, DNA digests were precipitated with ethanol and separated in 1.4% agarose gels. Bands were cut out, the agarose was dissolved in 4 ml of water at 120°C, and 10 ml of toluene-Triton X-100 (2:1) scintillator was added for counting in the gel phase.

RESULTS

Base composition and molecular weight of *H. ateles* DNA. The densities of both intact

H. ateles DNA molecules and fragmented DNA were determined independently in the analytical ultracentrifuge.

DNA from *H. ateles* 810 and 73 was sheared by rapid passage of purified DNA through a 26-gauge, one-half-inch (ca. 12.5 mm) syringe needle. The DNA fragments banded at two widely different densities. Figure 1B shows the density profile of fragmented DNA from strain

FIG. 1. Photoelectric scanings of *H. ateles* DNA centrifuged to equilibrium in CsCl in a Spinco model E analytical ultracentrifuge. (A) DNA of gently lysed particles of partially purified *H. ateles* 73; (B) same DNA as in (A), but mechanically sheared before centrifugation; and (C) sheared *H. ateles* DNA (strain 810) with *E. coli* DNA and herpes simplex virus type 2 DNA added as internal density markers.

73. A class of heavier DNA (H-DNA) comprised about 40%, and a second class (L-DNA) comprised about 60% of the total DNA.

The absolute density values of H- and L-DNA in CsCl were determined by centrifuging a mixture of fragmented DNA from *H. ateles* and *Escherichia coli* (1.710 g/ml) and DNA from herpes simplex virus type 2 (1.727 g/ml) (21) (Fig. 1C). H-DNA of both *H. ateles* strains had a density of 1.7330 to 1.7333 g/ml, and L-DNA had a density of 1.6967 g/ml (Table 1). This corresponds to 75% G+C for H-DNA and 38% G+C for L-DNA, provided that *H. ateles* DNA does not contain unusual bases.

Thus, the genomes of *H. ateles* and *H. saimiri* were similar with regard to an unusual heterogeneity in base composition. H- and L-DNA from *H. ateles*, however, had significantly higher G+C contents than the corresponding *H. saimiri* DNA fragments analyzed in a parallel run with the same density markers.

When the density of *H. ateles* DNA was analyzed under conditions of minimal shearing, most of the DNA was found in one band at an intermediate density (Fig. 1A). The buoyant density of this M-DNA from *H. ateles* was 1.7061 to 1.7064 g/ml (Table 1). Figure 2A shows the density profile after centrifugation of intact isolated M-DNA from both *H. ateles* strains and from *H. saimiri* by using adenovirus type 2 DNA (57% G+C) as a reference point (14). After hydrodynamic shearing of M-DNA from *H. ateles* (and from *H. saimiri*), the band of M-DNA disappeared and two new bands appeared, one with the density of H-DNA and one with that of L-DNA (Fig. 2B). Hence, the majority of DNA molecules from *H. ateles* virions consisted of H- and L-DNA in linear association similar to *H. saimiri* M-DNA. If M-genomes with an average G+C content of 47.5% are built up from H-DNA (75% G+C) and L-DNA (38% G+C), then M-DNA of both strains of *H. ateles* contain an estimated 26% H-DNA and 74% L-DNA.

In all analytical isopycnic bandings performed under conditions of minimal shearing, *H. ateles* DNA showed a small H-DNA peak at 1.7330 to 1.7333 g/ml (Fig. 1A). This may be, in part, due to unavoidable fragmentation of DNA. However, it suggested the presence of H-genomes in analogy to *H. saimiri* H-genomes (see below).

Scanning these CsCl gradients in a Spinco model E analytical ultracentrifuge revealed a variable shoulder at the position of L-DNA (Fig. 1A and 2A), possibly due to fragmentation or to a slight contamination with cellular DNA. However, the possibility that unit-length molecules consisting exclusively of *H. ateles* L-DNA existed could not be ruled out, although such

TABLE 1. Buoyant densities in CsCl and G+C content of <i>H. ateles</i> and <i>H. saimiri</i> DNA								
Strain	H-DNA		M-DNA		L-DNA		% H-DNA in M-DNA	% L-DNA in M-DNA
	ρ (g/ml)	% G+C	ρ (g/ml)	% G+C	ρ (g/ml)	% G+C		
<i>H. ateles</i>								
810	1.7330 $\pm$ 0.0005	74.5	1.7061 $\pm$ 0.0003	47.0	1.6967 $\pm$ 0.0007	37.5	25.8	74.2
73	1.7333 $\pm$ 0.0008	74.8	1.7064 $\pm$ 0.0008	47.3	1.6967 $\pm$ 0.0007	37.5	26.4	73.6
<i>H. saimiri</i>								
11 ^a	1.7292 $\pm$ 0.0006	70.6	1.7045 $\pm$ 0.0005	45.4	1.6951 $\pm$ 0.0002	35.8	28	72

^a See reference 9.

FIG. 2. Photoelectric scanings of isolated M-DNA from *H. ateles* 810 and 73 and *H. saimiri* after centrifugation with DNA from adenovirus type 2. (A) (1 to 3) Conditions avoiding fragmentation of DNA molecules. (B) (1 to 3) Same DNA as in (A), but mechanically sheared before centrifugation.

molecules have never been observed during partial denaturation mapping of M-genomes (see below). The lengths of 58 unsheared M-DNA molecules isolated from *H. ateles* virions were measured in an electron microscope by using bacteriophage PM2 DNA as an internal size marker (25). A histogram of the length distribution of

the molecules is shown in Fig. 3. The average molecular weight of the 59 molecules measured was found to be $89.8 \pm 8.5 \times 10^6$. Length variations exceeded by far the standard deviation of the method, which would be about 1% (1).

Another 15 molecules shorter than $35 \mu\text{m}$ were regarded as fragments and were not included in the histogram or the molecular-weight estimation.

Arrangement of H- and L-DNA sequences within *H. ateles* genomes. Heavy and light sequences in M-DNA of *H. ateles* were localized by using the different stabilities of G-C and adenine-thymine base pairs under melting conditions. Figure 4 shows an electron micrograph of an M-DNA molecule partially denatured by sodium perchlorate and formaldehyde. The central region of the molecule was almost completely denatured and apparently contained only L-DNA sequences. Both terminal stretches remained duplex and did not display any melted region, thus indicating that they contained a high concentration of uniformly distributed G-C pairs.

The lengths of remaining duplex DNA at each end of an M-DNA molecule varied between <1 and $17 \mu\text{m}$ (Fig. 5A). *H. ateles* M-genomes with a long duplex stretch at one side had a short duplex region at the other side, resulting in a more constant length of the sum of the remaining duplex areas in a given molecule (Fig. 5B). However, its length still varied between 8 and $18 \mu\text{m}$. Thus, different DNA molecules appeared to have various lengths of H-DNA stretches at their ends.

Partially denatured molecules used for these

FIG. 3. Histogram of the contour lengths of 58 M-DNA molecules isolated from *H. ateles* virions.

measurements had short duplex areas at defined positions in the central or light portion of the molecules. When these areas were used to orient the molecules, it was found that the longer duplex terminal stretches might be located at either side of the molecule. It was therefore concluded that the terminally located stretches of H-DNA could be randomly distributed over the ends, similar to *H. saimiri* genomes (1).

DNA was extracted from *H. ateles* particles under conditions avoiding mechanical shearing. The material banding in CsCl between 1.730 and 1.735 g/ml was subjected to the same partial denaturation conditions as was the M-DNA molecule shown in Fig. 4. In this heavy-density range, long molecules that remained duplex over their entire length (Fig. 6) were found. This indicated that a number of *H. ateles* particles contained long molecules consisting of H-DNA only; it was in good accordance with the finding of H-DNA peaks in scannings of analytical density gradients of nonfragmented *H. ateles* DNA (see above), and it suggested again that *H. ateles* virions contained H-genomes analogous to those of *H. saimiri*.

Genetic complexity of H-DNA from *H. ateles*. H-DNA of *H. saimiri* consists of highly repetitive sequences, as shown by reassociation kinetics (9), and cleavage with restriction endonucleases (1). Several restriction endonucleases (*Bam*HI, *Eco*RI, *Hsu* I, *Kpn* I, *Pst* I, *Sac* I, *Sac* II, *Sal* I, *Sma* I, and *Xho* I) have been used for a structural analysis and strain comparison of *H. ateles* DNA (C. Mulder and B. Fleckenstein, unpublished data). Of these, only endonucleases R·*Sac* II, *Sma* I, and *Pst* I were found to cleave H-DNA of both *H. ateles* strains. These H-DNAs were cleaved into multiple fragments that fell into a small number of size classes. The combined sizes of these fragment classes enabled us to estimate the genetic complexity of these sequences.

Figure 7 shows the separation of endo R·*Sma* I fragments of *H. ateles* H-DNA in 5% acrylamide.

After cleavage of the DNA from *H. ateles* 810, four distinct size classes of DNA fragments were identified with molecular weights of 520,000, 190,000, 140,000 and 75,000 (fragments A to E). Fragments with a molecular weight of less than 30,000 would not have been detected under these conditions. Endo R·*Sma* I fragments of adenovirus type 2 DNA and of *H. saimiri* 11 H-DNA, endo R·*Hind*II + III fragments of simian virus 40 DNA (2), and *Hind*III fragments of bacteriophage PM2 were used as size markers (1).

An attempt was made to estimate the sizes of the *Sma* I fragments of H-DNA independ-

FIG. 4. Electron micrograph of a partially denatured molecule of *H. ateles* M-genome. The arrows indicate the transitions between duplex H-DNA and the partially denatured L-region. The small circular molecules are PM2 DNA added as size markers.

FIG. 5. Histograms showing the lengths of duplex termini from 20 *H. ateles* M-DNA molecules. (A) Each individual terminus is represented. (B) The sum of the lengths of both ends from one molecule is compiled.

ently by determining the relative amounts of label present in each band after electrophoresis in 1.5% agarose of *H. ateles* H-DNA after cleavage with endo R·*Sma* I. The results (Table 2) indicated that the fragments migrating in the third band had about twice the amount of ^3H expected for a fragment that size, suggesting that this band contained two fragments (C and D) of identical size. The fourth band (fragment E) also contained an excess of ^3H counts, about 1.5 times the calculated amount. This could be caused by a relatively higher A+T content of this fragment or by contamination with degradation products of larger *Sma* I fragments. However, it is also possible that this band represented two fragments with a relatively low A+T content, thus containing less ^3H .

Cleavage of H-DNA from *H. ateles* 73 by endo R·*Sma* I resulted in fragments present in equimolar amounts (Fig. 7). The molecular weights of these fragments were estimated as 460,000, 170,000, 130,000, and 115,000 (fragments A to D). Some gels revealed a fifth small fragment (E) with a molecular weight of 35,000 (not shown in Fig. 7). Figure 7 also revealed a minor band corresponding to a molecular weight of 400,000; however, no other batches of H-DNA from *H. ateles* 73 yielded any minor bands; therefore this was presumably due to a heterogeneity of this DNA preparation.

Base sequence homology between DNA of the two *H. ateles* strains. A series of cross-reassociation experiments was done to determine to what extent the L-DNA regions of *H. ateles* 810 and 73 were homologous, and similar

hybridizations were done with their H-DNAs. It was consistently found that labeled viral DNA reassociated with an excess of unlabeled heterologous DNA to the same extent as with homologous DNA.

The L-DNA homoduplexes and heteroduplexes formed in these reactions were used to define the genetic relationship between the two *H. ateles* strains from an estimation of their base-pair divergence.

L-DNA hybrids consisting of labeled strain 73 DNA and unlabeled DNA from OMK cells infected with strain 810 were isolated by hydroxyapatite chromatography. The melting temperature (T_m) of these heterologous hybrids was compared with that of homologous hybrids. These homologous hybrids were formed by reassociation of purified ^3H -labeled strain 73 DNA and unlabeled DNA from strain 73-infected OMK cells sheared to equal sizes. Heterologous hybrids showed a relative decrease in T_m of 3.4°C (Fig. 8). By using the formula of Ullman and McCarthy ($\Delta T_m = 1.5^\circ\text{C}/1\%$ mismatching) (31), this decrease corresponded to about 2.3% mismatching in the hybrids of L-DNA from *H. ateles* 810 and 73.

Analogous experiments were done with ^3H -labeled H-DNA from strain 73 and unlabeled total DNA of OMK cells lytically infected with *H. ateles* 810 and 73 (Fig. 9). The difference in T_m of homoduplexes versus heteroduplexes was 5°C.

Base sequence homologies between L-DNA of *H. ateles* and *H. saimiri*. The relationship between the L-DNA of *H. ateles* and *H. saimiri* was estimated by cross-hybridization. Figure 10A shows the reassociation of ^3H -labeled L-DNA from *H. ateles* 810 in the presence of an excess of unlabeled L-DNA from *H. saimiri*. Extrapolation of the second phase of the bimodal reassociation curve of Fig. 10A indicated that the reassociation of *H. saimiri* DNA would be accelerated to a C_0/C_t value of about 1.55 in the absence of self-hybridization. This would correspond to about 35% homology (33) between *H. ateles* and *H. saimiri* DNA.

The inverse experiment with [^3H]thymidine-labeled L-DNA from *H. saimiri* and an excess of unlabeled DNA from 22CM37 cells revealed the same degree of homology between L-DNA of these viruses (Fig. 10B). 22CM37 cells are a virus-producing cell line obtained by in vitro transformation of marmoset lymphocytes (7).

L-DNA hybrids formed by hybridization of ^3H -labeled *H. saimiri* DNA and an excess of unlabeled *H. ateles* DNA were isolated by chromatography on hydroxyapatite. The melting temperatures of these heteroduplexes was decreased by 13.5°C, compared to that of homol-

FIG. 7. Gel electrophoresis of *Sma* I fragments of H-DNA from *H. ateles* 810 and 73 and from *H. saimiri* 11. *Sma* I fragments of adenovirus type 2 DNA were run as size markers in this gel (*Sma* K fragment, 380,000 molecular weight; *Sma* L fragment, 140,000 molecular weight; and *Sma* M fragment, 110,000 molecular weight).

TABLE 2. Restriction fragments obtained by cleavage of ^3H -labeled DNA from *H. ateles* 810 with *endo* R · *Sma* I

Fragment	Mol wt	dpm	% Total radio-activity	Molar-ity
A	520,000	1,790	43.5	1
B	190,000	740	18.0	1
C + D	140,000	1,136	27.5	2
E	75,000	455	11.0	1

ogous hybrids (Fig. 11). This might indicate an average of about 9% unmatching in the hybridizing fractions of H-DNA from *H. ateles* and *H. saimiri*. However, the heteroduplexes appeared to melt over a considerably broader temperature range than the homoduplexes; this indicated a heterogeneity in the extent of mismatching of base pairs.

Base sequence homologies between H-DNA of *H. ateles* and *H. saimiri*. Cross-hybridizations with H-DNA showed a lower degree of homology in the repetitive sequences of these viruses. ^3H -labeled H-DNA from *H. saimiri* was hybridized with an excess of *H. ateles* or *H. saimiri* DNA, respectively. Ten percent of ^3H -

labeled *H. saimiri* H-DNA did hybridize with unlabeled DNA extracted from OMK cells lytically infected with *H. ateles* 810 (Fig. 12).

For determination of base sequence divergence in the cross-hybridizing fractions of H-sequences from *H. ateles* and *H. saimiri*, annealing of 60 ng of ^3H -labeled H-DNA from *H. saimiri* (specific activity, 670,000 dpm/ μg) per ml was carried out for 1 min in the presence of 100 μg of unlabeled DNA from *H. ateles*-transformed 22CM37 cells per ml. Based on the kinetics of hybrid formation (Fig. 13), it was expected that at this time about 40% of hybridized labeled *H. saimiri* H-DNA was in heterologous and 60% in homologous hybrids. Correspondingly, a biphasic melting curve was obtained. Forty percent of labeled DNA, apparently representing *H. saimiri*-*H. ateles* H-DNA hybrids, was converted to single-stranded DNA at temperatures below 76°C, whereas the T_m of homologous hybrids was 96°C. This indicated that the

FIG. 8. Melting profiles of *H. ateles* L-DNA homoduplex and heteroduplex molecules. ^3H -labeled L-DNA from *H. ateles* 73 (320,000 dpm/ μg , 125 ng/ml) was hybridized with 100 μg of unlabeled DNA from OMK cells lytically infected with *H. ateles* 73 or 810 per ml. Double-stranded molecules were isolated by hydroxyapatite chromatography. The fraction containing duplex molecules was diluted to a final concentration of 0.14 M sodium phosphate buffer, and the DNA was denatured at increasing temperatures between 62.5 and 87.5°C. The T_m of homoduplex molecules was at 81.2°C (○), and that of heteroduplexes was at 77.8°C (●).

FIG. 6. Electron micrograph of a DNA molecule from the high-density range ($\rho = 1.730 - 1.735 \text{ g/ml}$) subjected to the same denaturing conditions as was the molecule shown in Fig. 2. The contour length is 41.5 μm . As an internal control for the denaturation, DNA from bacteriophage PM2 (G+C 43%) was included before denaturation. After denaturation, additional PM2 DNA was added as a size marker. PM2 DNA is taken to have a molecular weight of 6.4×10^6 (25).

cross-hybridizing sequences in H-DNA of *H. ateles* and *H. saimiri* diverged in more than 13% of their base pairs.

DISCUSSION

The biological and oncogenic properties of *H. ateles* and *H. saimiri* show several similarities. Correspondingly, their DNAs share two uncom-

mon features: (i) an extreme heterogeneity in base composition and (ii) highly repetitive sequences.

Two *H. ateles* DNA species have been iden-

FIG. 9. Melting profiles of *H. ateles* H-DNA homoduplex and heteroduplex molecules. ^3H -labeled H-DNA from *H. ateles* 73 (130,000 dpm/ μg , 500 ng/ml) was hybridized with 100 μg of unlabeled DNA from OMK cells lytically infected with *H. ateles* 73 or 810 per ml. The T_m of heterologous hybrids (○) was found to be 5°C lower than that of homologous hybrids (●).

FIG. 11. Melting curve of DNA hybrids formed between ^3H -labeled *H. saimiri* L-DNA (96 ng/ml; specific activity, 1.45×10^6 dpm/ μg) and 1,500 μg of unlabeled DNA from *H. ateles*-transformed 22CM37 cells (●) per ml. The biphasic melting curve reflects that about 20% of the hybrids formed are due to self-annealing of ^3H -labeled *H. saimiri* DNA. Melting of homologous hybrids formed between ^3H -labeled *H. saimiri* L-DNA (64 ng/ml; specific activity, 1.45×10^6 dpm/ μg) and 50 μg of DNA from *H. saimiri*-infected OMK cells (○) per ml.

FIG. 10. Cross-hybridization of *H. ateles* and *H. saimiri* L-DNA. (A) Reassociation of ^3H -labeled L-DNA of *H. ateles* 810 (100 ng/ml; 304,000 dpm/ μg) with 10 μg of unlabeled *H. saimiri* L-DNA (●). Self-annealing of ^3H -labeled *H. ateles* L-DNA (○). (B) Reassociation of ^3H -labeled L-DNA from *H. saimiri* (64 ng/ml; 1.45×10^6 dpm/ μg) with 2 mg of DNA of *H. ateles*-transformed lymphoid cell line 22CM37 per ml (●), 2 mg of calf thymus DNA per ml (○), or 50 μg of DNA from *H. saimiri*-infected cells per ml supplemented with calf thymus DNA to a final concentration of 2 mg/ml (□). (C_0/C_t = dpm in total DNA/dpm in single-stranded DNA.)

FIG. 12. Reassociation of ^3H -labeled H-DNA from *H. saimiri* 11 (60 ng/ml; specific activity, 670,000 dpm/ μg) with 200 μg of unlabeled DNA from *H. ateles* 810-infected OMK cells per ml ($\bullet$), 100 μg of unlabeled DNA from *H. saimiri* 11-infected OMK cells per ml ($\square$), and calf thymus DNA ($\circ$). In all reactions, the DNA concentration was adjusted to 1 mg/ml by adding calf thymus DNA. (C_0/C_t = dpm in total DNA/dpm in single-stranded DNA.)

FIG. 13. Melting profile of H-DNA hybrids formed between ^3H -labeled *H. saimiri* H-DNA (60 ng/ml; specific activity, 670,000 dpm/ μg) and 100 μg of DNA from *H. ateles*-transformed 22CM37 cells per ml ($\bullet$) and between ^3H -labeled *H. saimiri* H-DNA (60 ng/ml; specific activity, 670,000 dpm/ μg) and 100 μg of DNA from *H. saimiri*-infected OMK cells per ml ($\circ$). The biphasic melting curve ($\bullet$) is because only about 40% of the hybrids formed are heteroduplexes.

tified by analytical centrifugation: (i) L-DNA with 38% G+C and (ii) H-DNA with 75% G+C. As shown by partial denaturation, these two types of DNA sequences are assembled to at

least two types of double-stranded molecules: (i) M-genomes and (ii) H-genomes with molecular weights of approximately 90×10^6 . H-genomes consist of H-DNA exclusively. M-genomes contain an average of 74% L-DNA inserted between terminal H-DNA sequences that represent an average of 26% of the molecular length (Fig. 14).

H-DNA is highly repetitive. M-genomes contain about 25 identical repeat units of H-DNA. DNA sequences in H-genomes are approximately 100-fold reiterated, and such molecules are probably defective genomes.

Thus, the structural organization of *H. ateles* genomes is similar to that of *H. saimiri*. However, two differences were observed: (i) H-DNA, as well as L-DNA, of *H. ateles* had a significantly higher G+C content than the corresponding sequences of *H. saimiri* and (ii) the relative amount of H-DNA in M-genomes of *H. ateles* 810 and 73 was found to be lower than the percentage of H-DNA in M-genomes of all *H. saimiri* strains tested so far.

Until now, no other herpesviruses have been found with comparable genome structures. Herpes simplex DNA from infectious virions was shown to contain inverted repeat units (15, 29), but not highly repetitive sequences. Also Epstein-Barr virus differs in the structural organization of its genome from *H. saimiri* and *H. ateles*, since a pronounced heterogeneity in base composition and highly repetitive sequences has not been found in the Epstein-Barr virus strains isolated from P3HR-1 and B95-8 cells, respectively (26, 30; G. W. Bornkamm, B. Fleckenstein, F.-J. Werner, and C. Mulder, unpublished data).

Both L- and H-DNA sequences of *H. ateles* 810 and 73 are closely related; heteroduplex molecules showed a low degree of mismatching (about 2.4% in L-DNA and 3.3% in H-DNA), evident from slight depressions of T_m values in relation to the melting profiles of homoduplexes.

The estimation of base pair divergence in *H.*

FIG. 14. Scheme of the predominant types of DNA molecules isolated from *H. ateles* particles showing M-genome build up from an internal L-region inserted between repetitive H-DNA termini of high G+C content and H-genomes consisting of H-DNA repeat units.

ateles DNA by using the formula of Ullman and McCarthy (31) may be inaccurate. The correlation ($\Delta T_m = 1.5^\circ\text{C}/1\%$ mismatching) was mainly obtained from results with chemically induced point mutations. The possibility exists that other relations would be valid for the high and low G+C DNA of related viruses.

Digestion of *H. ateles* H-DNA with endonuclease R·*Sma* I and with other restriction enzymes (*Hinf*I, *Pst* I, *Sac* I, and *Taq* I) (Mulder and Fleckenstein, unpublished data) resulted in distinct cleavage patterns for the two strains 810 and 73. It allowed us to differentiate *H. ateles* strains by the cleavage specificity; similarly, three *H. saimiri* strains could be typed by a series of restriction endonucleases (Fleckenstein and Mulder, manuscript in preparation).

The endo R·*Sma* I-C/D band of H-DNA from *H. ateles* 810 showed a twofold molarity. Two possibilities may account for that: (i) The C/D band consists of two different fragments that accidentally have the same size. In this case, the complexity of *H. ateles* H-DNA would correspond to a molecular weight of 1,060,000. (ii) The twofold molarity is due to an internal repeat within the *H. ateles* H-DNA repeat unit. In this case, the complexity of *H. ateles* H-DNA should have a molecular weight of less than 930,000. The latter explanation seems to be more plausible since other endonucleases also generated bands of twofold molarity (unpublished data).

L-DNA from *H. ateles* and *H. saimiri* was found to be about 35% homologous, and their H-DNAs showed homology in only 10% of their sequences under the hybridization conditions used. The base sequence divergence in the cross-hybridizing parts of L-DNA is approximately 9%, whereas that of H-DNA is even higher. Thus, with regard to the high-sequence homology between the *H. ateles* strains, the pronounced sequence divergence between the *H. ateles* strains and *H. saimiri* supports the view that these viruses should be regarded as distinct but related entities within the herpesvirus family.

The homologies found between *H. ateles* and *H. saimiri* DNA suggest that the viruses have evolved from a common ancestor. Conceivably, the ancestor virus had an L-region from which the L-DNA sequences of *H. ateles* and *H. saimiri* were derived. Two possibilities can be deduced for the origin of repetitive DNA: (i) Heavy sequences of *H. ateles* and *H. saimiri* may have come from a common sequence present in the ancestor virus. The higher divergence of H-DNA sequences of both viruses may indicate a lower need to preserve the H-DNA sequences in evolution. (ii) Alternatively, the H-DNA sequences

may have been picked up independently by both viruses from similar cellular DNA sequences, thus explaining the different rate of sequence divergence for H- and L-DNA sequences. Attempts are in progress to search for homologies between H-DNA and cellular DNA of several nonhuman primate species.

ACKNOWLEDGMENTS

We thank Harald zur Hausen for helpful discussions and advice. We greatly appreciate the excellent technical assistance of Ingrid Müller, and we thank A. Pühler and W. Schöffel for helping with analytical centrifugations.

This work was supported by the Deutsche Forschungsgemeinschaft, Bad Godesberg (SFB118 "Methodenforschung zur Früherkennung des Krebses"), by Public Health Service Cancer Research Emphasis Grant R01 CA 22271-C1 and contract NO1 CP 33390 from the Virus Cancer Program of the National Cancer Institute, and by grant VC-197 of the American Cancer Society (to C.M.). L.A.F. is a scholar of the Leukemia Society of America, Inc.

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