JOURNAL OF VIROLOGY, Nov. 1983, p. 377-383
0022-538X/83/110377-07$02.00/0
Copyright © 1983, American Society for Microbiology
Vol. 48, No. 2

Virus-Specific Transcription in a *Herpesvirus saimiri*-Transformed Lymphoid Tumor Cell Line

ELISABETH KNUST,† WALTER DIETRICH, BERNHARD FLECKENSTEIN, AND WALTER BODEMER*

Institut für Klinische Virologie, Universität Erlangen-Nürnberg, D-8520 Erlangen, West Germany

Received 15 March 1983/Accepted 25 July 1983

Herpesvirus saimiri-transformed lymphoid tumor cell lines contain nonintegrated covalently closed circular viral DNA molecules in high multiplicity. One of those cell lines, 1670, carries large viral DNA circles (202 kilobase pairs) with two stretches of repetitive DNA (70.8% G + C) that are interspersed between two segments of unique DNA (36% G + C). Since it was not known if there is any viral gene expression in *H. saimiri*-transformed cells, we initiated a study of transcription in cell line 1670. cDNA was generated by reverse transcription of cellular RNA and hybridized with cloned virion DNA fragments. The experiments indicated that appreciable transcription is restricted to a single segment of unique DNA. This sequence is present once only in the circular viral DNA and corresponds to unique DNA between map units 0.89 and 0.93 of virion DNA. By Northern blot hybridizations with labeled cloned probes of virion unique DNA, one predominant virus-specific polyadenylated transcript of, at most, 2.7 kilobases could be detected in tumor cell line 1670. The direction of transcription was determined by hybridization with randomly primed cDNA and, in parallel, with oligodeoxythymidylate-primed cDNA probes. Apparently, the patterns of virus-specific RNA synthesis in the *H. saimiri*-transformed cells are clearly distinct from the transcription program in other herpesvirus transformation systems analyzed before.

Herpesvirus saimiri, a natural inhabitant of squirrel monkeys (*Saimiri sciureus*), causes malignant tumors of the lymphatic system in a number of New World primate species and rabbits (7, 16, 29). Several transformed lymphoid tumor cell lines have been derived from lymphomas of marmoset monkeys (21, 31); all *H. saimiri*-transformed cells investigated so far reveal surface markers of T lymphocytes (15). The tumor cell lines produced infectious virus initially after explantation into culture. Some of these lines, propagated in vitro for several years, have ceased producing virus: no infectious *H. saimiri* could be isolated from those nonproducer cell lines by cocultivation with permissive monolayer cells.

The genome organization of *H. saimiri* is different from that of all herpesviruses characterized before (20). Most intact DNA molecules (M-DNA) extracted from purified virus particles are linear duplex molecules of 145 to 170 kilobase pairs (kbp), consisting of about 30% repetitive DNA (H-DNA) (70.8% G + C) and 70% unique DNA (L-DNA) (36% G + C) (17). The L-DNA region (112 kbp) is inserted between two variable stretches of H-DNA. The H-DNA consists of identical repeat units (each 1,444 base pairs [bp]) in tandem orientation (A. Bankier, R. Baer, B. G. Barrell, F. Colbére-Garapin, W. Dietrich, E. Knust, and B. Fleckenstein, unpublished data). Isolated M-DNA from virus particles is infectious in cell culture (17) and oncogenic upon transfection in marmoset monkeys (18). *H. saimiri*-transformed lymphoid tumor cell lines contain covalently closed circular viral DNA molecules in high copy number (21, 24). A few lymphoid cell lines have been characterized in more detail with regard to the structure of their nonintegrated viral DNA. All circular viral genomes of nonproducer cell lines analyzed by partial denaturation mapping in the electron microscope have large deletions of their L-DNA region (10, 24, 40, 41). The cell line 1670, derived from the spleen of a white-lip marmoset (19) contains about 210 viral genome equivalents per cell. The circular molecules of this cell line (202 kbp) are significantly larger than virion DNA (40). They contain a long and a short region of L-DNA (about 90 and 52 kbp, respectively), separated by two segments of H-DNA (35 and 26

† Present address: Institut für Entwicklungsphysiologie der Universität Köln, D-5000 Köln 41, West Germany.

kbp). The short L-DNA region is a subset of the longer L-DNA; the long L-segment differs from virion DNA by an internal deletion of ca. 21 kbp (10, 41) (see Fig. 1).

In contrast to high viral genome copy numbers, no *H. saimiri*-specific protein has been detected so far in transformed cells (19). This led to the hypothesis that the low extent of gene expression may be a consequence of the high degree of methylation in persisting viral DNA (12). Desrosiers (11) has localized a few specifically unmethylated CG sites within the L-DNA of fresh tumor cells and the lymphoid tumor cell line 1670. We began to study transcription of 1670 cells to determine whether there is any viral gene expression in *H. saimiri*-transformed cells and, if so, whether transcription of certain genomic regions may be correlated with detectable patterns of hypomethylation.

MATERIALS AND METHODS

Cell culture. The lymphoid cell line 1670 was obtained from L. A. Falk (27) and propagated in our laboratory continuously. The cells were grown as suspension cultures in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and 350 μg of glutamine, 100 U of penicillin, and 100 μg of streptomycin per ml.

Preparation of RNA. All buffers and glassware were autoclaved before use. The initial steps in RNA preparation were performed at 0 to 4°C. For isolation of total cellular RNA, cells were collected by low-speed centrifugation at 1,500 rpm for 5 min and washed once with ice-cold phosphate-buffered saline (0.14 M NaCl, 2.5 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4 [pH 7.0]) containing 2 μg of polyvinylsulfate per ml. Cell pellets (ca. 3×10^8 cells) were lysed in 6 ml of guanidinium isothiocyanate buffer (5 M guanidinium isothiocyanate, 50 mM lithium citrate, 0.1% lithium lauryl sulfate, 0.1 M β-mercaptoethanol [pH 6.5]) (9). RNA was centrifuged through a 4-ml cushion of 5.7 M CsCl in 0.1 M EDTA in a Spinco SW41 rotor for 20 to 24 h at 20°C and 30,000 rpm (4, 22). After centrifugation, the material above the gelatinous RNA pellet was carefully removed. The pellet was drained, and the bottom of the tube was cut off. The RNA was dissolved in 0.5 to 1 ml of water and precipitated in 0.2 M potassium acetate or NaCl with 2.5 volumes of ethanol at −20°C. The precipitated RNA was collected by centrifugation in an HB-4 rotor (Ivan Sorvall Inc.) at 12,000 rpm for 1 h or in a Beckman microfuge (type B) for 20 min at maximum speed.

RNA was fractionated by three cycles of oligodeoxythymidylate (oligo[dT])-cellulose chromatography (Bethesda Research Laboratories) (3). The polyadenylated (poly[A]$^+$) RNA was precipitated in 0.2 M potassium acetate with 2.5 volumes of ethanol at −20°C.

cDNA synthesis and Southern blot hybridization. DNA complementary to poly(A)$^+$ RNA was synthesized with avian myeloblastosis virus reverse transcriptase (J. W. Beard, Life Sciences, Inc.) modified after several published procedures (1, 5, 39). The reaction mixture (50 to 100 μl) contained 4 to 10 μg of RNA, 0.4 mM each dCTP, dGTP, and dTTP, 0.04 mM dATP, 150 μCi of [α-^{32}P]dATP (800 Ci/mmol; New England Nuclear Corp.), 50 mM Tris (pH 8.3), 40 to 140 mM KCl, 7 to 10 mM $MgCl_2$, 7 to 10 mM dithioerythritol, and 20 to 40 U of reverse transcriptase. The reaction was primed with 5 μM oligo(dT)$_{10}$ (Boehringer Mannheim Corp.) or calf thymus DNA digested by DNAse I (37). After 2 h of incubation at 37°C, the reaction was stopped by adding EDTA to give a final concentration of 20 mM. The DNA was extracted with an equal volume of phenol; salmon sperm DNA was added to a final concentration of 1 mg/ml, and the nucleic acids were precipitated with 2.5 volumes of ethanol at −20°C. Nucleic acids were collected by centrifugation in a Beckman microfuge (type B) for 20 min at maximum speed. The RNA was hydrolized in 0.3 M NaOH and 1 mM EDTA and incubated at 65°C for 20 min. The mixture was chilled on ice and diluted in hybridization buffer. The average size of the cDNA was determined on 1.3% agarose gels and found to be equivalent to single-stranded DNA of 500 to 1,000 bases.

Cloned viral DNA was cleaved with restriction endonucleases as recommended by the manufacturers. The fragments were separated on 0.6% horizontal agarose gels and transferred to nitrocellulose filters (36). The filters were prehybridized in a solution of 0.1% Ficoll (Pharmacia Fine Chemicals), 0.1% polyvinylpyrrolidone (Sigma Chemical Co.) for 4 to 6 h at 65°C. The hybridization solution contained 50% formamide (purified with Amberlite MB-1 before use), 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.1% sodium dodecyl sulfate, 20 mM sodium phosphate, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 50 μg of polyadenylic acid (Sigma), and ^{32}P-labeled cDNA. Hybridization was carried out at 43°C for 15 to 18 h (35). The filters were washed several times with buffer containing 20 mM sodium phosphate, 0.1% sodium dodecyl sulfate, and decreasing concentrations of SSC (2× SSC to 0.1× SSC) for 3 h at 60°C. The dried filters were autoradiographed by using Kodak X-OMAT AR films and intensifying screens.

Nick-repair labeling of DNA and Northern blot hybridization. Labeling of DNA with [α-^{32}P]dATP followed the procedure described by Rigby (33) with minor modifications. The reaction mixture (250 μl) contained 50 mM Tris (pH 7.4), 10 mM $MgCl_2$, 0.02 mM each dGTP, dCTP, dTTP (Boehringer Mannheim), 30 ng of activated DNAase I per ml (Worthington Diagnostics), 22 U of DNA polymerase I (Boehringer Mannheim), 80 to 100 μCi of [α-^{32}P]dATP (800 Ci/mmol; New England Nuclear), and 1 μg of DNA substrate. After incubation for 60 min at 14°C, the radioactive product was isolated by Sephadex G-50 or 75 chromatography. A final specific activity of 1×10^8 to 2×10^8 cpm/μg of DNA was obtained.

RNA was denatured for 15 min at 56°C in 1 M glyoxal (freshly purified with Amberlite MB-1) in electrophoresis buffer (20 mM morpholinepropanesulfonic acid [Sigma], 5 mM sodium acetate, 1 mM EDTA) (28) and run in 1% agarose gels containing 2.2 M formaldehyde and 0.5 μg of ethidium bromide per ml. After electrophoresis, gels were treated with 50 mM NaOH for 1 h and neutralized with 10× SSC or 0.5 Tris-hydrochloride (pH 7.5) for 30 min; the RNA was transferred to nitrocellulose filters (SS-85, Schleicher and Schuell) in 20× SSC (38). The RNA was fixed by heat (4 to 10 h at 80°C), and the filters were

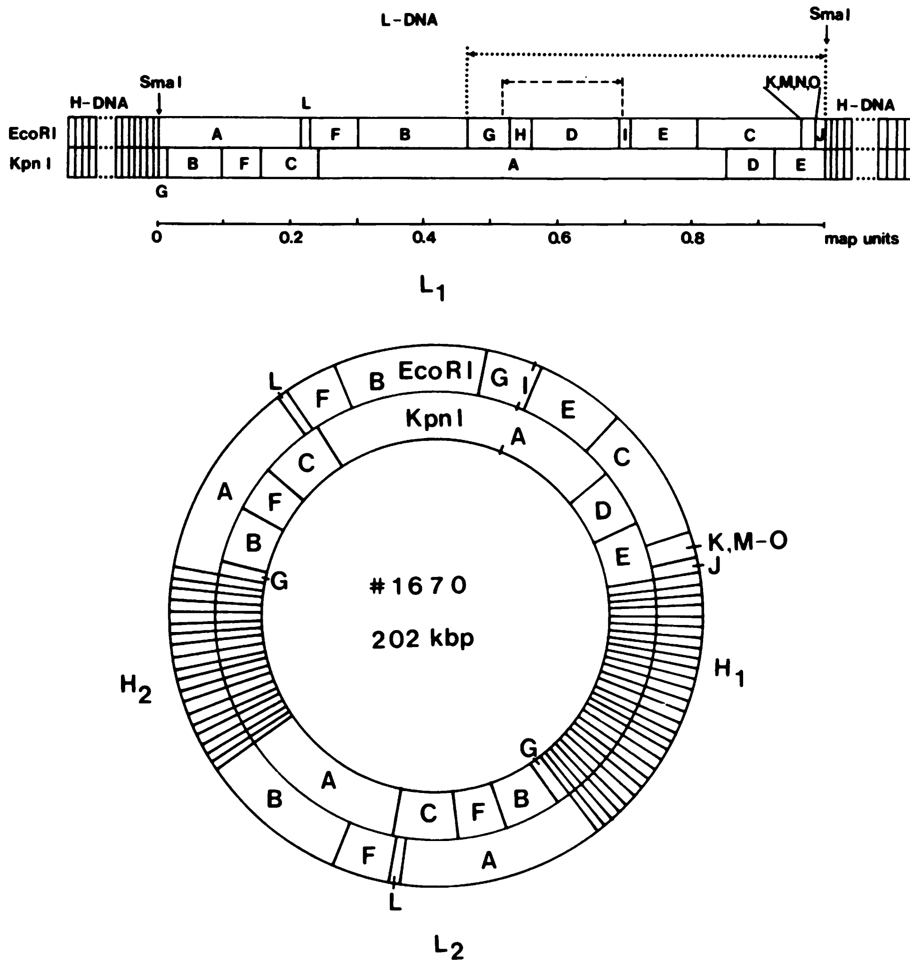

FIG. 1. Schematic representation of sequence arrangement in *H. saimiri* virion M-DNA and circular viral DNA of cell line 1670 (10, 40, 41). Virion L-DNA between interrupted lines is deleted in region L_1; virion L-DNA between dotted lines is missing in region L_2 in 1670 cells.

prehybridized for 4 to 6 h at 65°C in 2× SSC with 5× Denhardt solution (8) and 100 μg of yeast RNA per ml (Boehringer Mannheim). The hybridization solution contained 50% formamide, 5× SSC, 0.1% sodium dodecyl sulfate, 20 mM sodium phosphate, 1× Denhardt solution, and 100 μg of yeast RNA per ml. Nick repair-labeled DNA (ca. 2×10^5 to 5×10^5 cpm/cm^2 of filter) was hybridized to RNA for 48 to 60 h at 43°C. Washing procedures and autoradiography were done as described for cDNA hybridization.

RESULTS

Localization of predominantly transcribed L-DNA region. Initial experiments aimed at the detection of virus-specific transcription in 1670 cells were done by reassociation kinetics with ^{3}H-labeled M-DNA. This approach failed consistently. Similarly, it was not possible to find ^{3}H-labeled viral RNA in transformed cells by hybridization with purified virion DNA that was bound to membrane filters. To improve sensitivity, ^{32}P-labeled cDNA was synthesized by using poly(A)$^+$ RNA as the template and hybridized with cloned DNA fragments representing the entire *H. saimiri* genome. The internal *Eco*RI fragments B, D, E, F, G, H, I, K, L, M, and O were cloned in the plasmid vector pACYC 184. The *Eco*RI C fragment was inserted into bacteriophage lambda Charon 4A. The internal *Kpn*I fragments B, C, D, and F were cloned in the cosmid vector pJC 81, and the terminal *Kpn*I fragments E and G were inserted between the *Kpn*I and *Sma*I sites of the vector pWD7, which had been constructed for this purpose (E. Knust, S. Schirm, W. Dietrich, W. Bodemer, E. Kolb, and B. Fleckenstein, submitted for publication) (Fig. 1). H-DNA, cloned by Colbère-Garapin et al. in plasmid vector pAGO (6) was

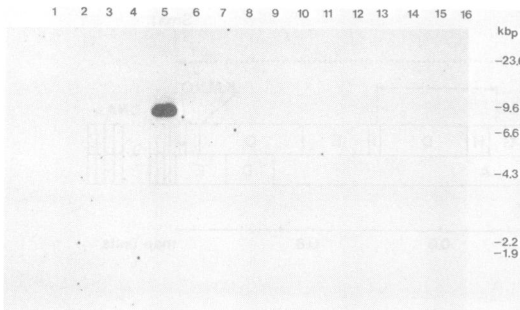

FIG. 2. Hybridization of randomly primed ^{32}P-labeled cDNA from 1670 cells with cloned fragments of virion H-DNA and L-DNA. Lanes: 1, *Kpn*I B fragment × *Kpn*I; 2, *Kpn*I F fragment × *Kpn*I; 3, *Kpn*I C fragment × *Kpn*I; 4, *Eco*RI B fragment × *Eco*RI; 5, *Eco*RI C fragment × *Hpa*II; 6, *Eco*RI D fragment × *Eco*RI; 7, *Eco*RI E fragment × *Eco*RI; 8, *Eco*RI F fragment × *Eco*RI; 9, *Eco*RI G fragment × *Eco*RI; 10, *Eco*RI H fragment × *Eco*RI; 11, *Eco*RI I fragment × *Eco*RI; 12, *Eco*RI K fragment × *Eco*RI; 13, *Eco*RI M fragment × *Eco*RI; 14, *Eco*RI O fragment × *Eco*RI; 15, *Kpn*I E fragment × *Eco*RI; 16, cloned H-DNA × *Taq*I.

included in the study. Oligo(dT)-primed cDNA strongly hybridized with the cloned *Eco*RI C fragment of *H. saimiri* L-DNA (Fig. 2). Identical patterns of hybridization, restricted to *Eco*RI fragment C, were observed by using cDNAs prepared from three batches of poly(A)$^+$ RNAs independently isolated from 1670 cells. There was no indication that cDNA made from RNA of uninfected owl monkey kidney (OMK) cells may possess any homology with viral DNA fragments (data not shown). If radioactive cDNA was synthesized from poly(A)$^+$ or poly(A)$^-$ RNA of 1670 cells with DNAase-digested calf thymus DNA as the random primer, no fragments other than *Eco*RI-C hybridized with the probe. The experiments indicated that abundant viral transcription in the transformed cells is restricted to the *Eco*RI C fragment of *H. saimiri* L-DNA. Randomly primed cDNA made from the poly(A)$^-$ RNA pool of 1670 cells hybridized with the cloned *Eco*RI C fragment to a very low extent (data not shown); apparently, the majority of viral transcripts in 1670 cells are poly(A)$^+$.

Size determination of the transcripts. To define the lengths of viral RNA molecules transcribed in 1670 cells, total cellular RNA was isolated and fractionated by oligo(dT) cellulose chromatography. After electrophoresis in denaturing 1% agarose gels, the RNA was transferred to nitrocellulose filters and hybridized with a nick repaired, ^{32}P-labeled cloned *Eco*RI C fragment. The majority of the poly(A)$^+$ transcripts were found in a single band at the position of ca. 2.7 kilobases (kb) of RNA, using rRNA of *Escherichia coli* and rRNA of 1670 cells as size markers (Fig. 3A). As shown in Fig. 3B, the same dominant class of poly(A)$^+$ RNA became apparent by Northern blot hybridization with the cloned *Kpn*I D fragment which is part of the *Eco*RI C fragment (Fig. 1). Some of the viral transcripts appeared as RNA of a smaller size (2.7 to 1.2 kb), most probably due to degradation. At present, it cannot be ruled out that smaller transcripts of the respective DNA region may occur in the transformed cells, either due to splicing at a low extent or secondary initiation or termination sites or both. No hybridization was found with poly(A)$^-$ RNA from 1670 cells, even with a 10-fold amount of RNA on the filters (Fig. 3A). Also, no homology was detected between cloned viral DNA and RNA from uninfected OMK cells (data not shown).

Detailed mapping of the transcribed region. A cleavage map of the *Eco*RI C fragment was derived for the restriction endonucleases *Hin*dIII, *Hpa*II, and *Kpn*I (Fig. 4). The *Eco*RI C fragment contains a single restriction site for restriction endonuclease *Hpa*II, and it is cleaved twice by restriction endonuclease *Kpn*I. The positions of nine restriction sites for restriction endonuclease *Hin*dIII were localized within the *Eco*RI C fragment; the fragment was partially cleaved with *Hin*dIII, and the order of *Hin*dIII fragments was established by Southern blot hybridization of a terminal *Hin*dIII fragment with partial digestion products. On the basis of this cleavage map, the viral sequences transcribed in 1670 cells could be localized more precisely. Hybridization of randomly primed cDNA was confined to the right of a 7.7 kbp *Eco*RI/*Hpa*II

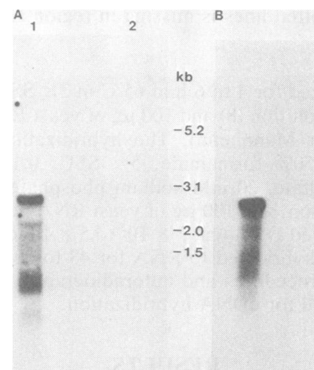

FIG. 3. Northern blot hybridization of poly(A)$^+$ RNA from 1670 cells with cloned fragments of *H. saimiri* L-DNA. (A) Lane 1, 20 μg of poly(A)$^+$ RNA; Lane 2, 200 μg of poly(A)$^-$ RNA hybridizing with the ^{32}P-labeled *Eco*RI C fragment. (B) 6 μg of poly(A)$^+$ RNA hybridizing with the labeled *Kpn*I D fragment. rRNA of 1670 cells (5.2 and 2.0 kb) and rRNA of *E. coli* (3.1 and 1.5 kb) were used as size markers.

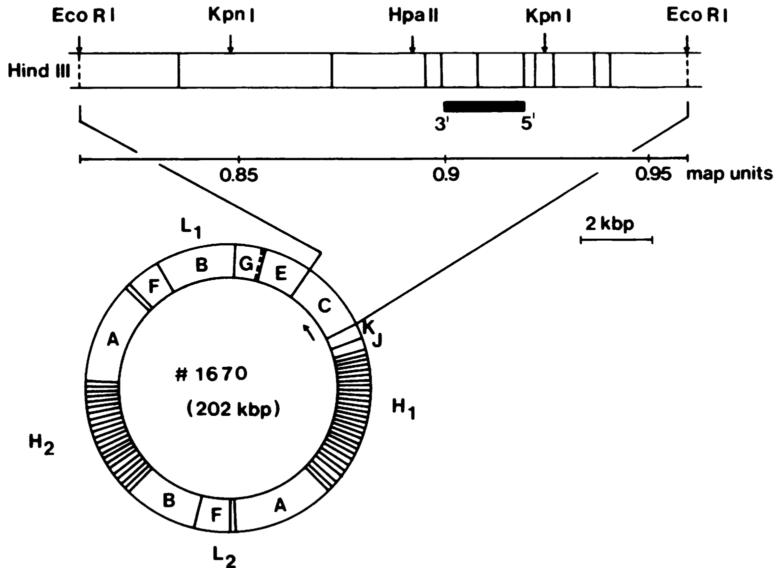

FIG. 4. Physical mapping of the DNA region transcribed in 1670 cells. Bar indicates size of the RNA and direction of transcription. Map units refer to virion DNA.

fragment. A series of hybridizations with oligo(dT)-primed cDNA indicated that the transcribed DNA is restricted to two adjacent *Hin*dIII fragments of 940 and 1,270 bp (Fig. 5a).

Direction of transcription. The orientation of the predominant transcript in 1670 cells was determined by comparing hybridization patterns with oligo(dT)-primed and randomly primed cDNA, a method similar to that described by Van Santen et al. (39). As oligo(dT)-primed cDNA primarily represents the 3'-terminal sequence of poly(A)$^+$ RNA, hybridization is expected to be less intense with DNA coding for the 5' end of the message. Oligo(dT)-primed cDNA hybridized with the *Hin*dIII fragment of 940 bp at a higher intensity than with the 1,270-bp fragment (Fig. 5A). In comparison, randomly primed cDNA hybridized with both fragments to about the same extent (Fig. 5b). This indicated that the 3' terminus of the transcript is encoded by the *Hin*dIII fragment of 940 bp; apparently, transcription starts in the 1,270-bp fragment or, possibly, in the adjacent 375-bp *Hin*dIII fragment.

DISCUSSION

The lymphoid tumor cell line 1670, like all other *H. saimiri*-transformed cells investigated so far, contains large, covalently closed circular viral DNA molecules in high multiplicity (24; R. C. Desrosiers and B. Fleckenstein, *in* G. Klein (ed.), *Advances in Viral Oncology*, in press). The circular molecules of 1670 cells (202 kbp) consist of two stretches of H-DNA and two L-DNA segments which are arranged in the same orientation. These L regions differ from virion L-DNA by the loss of internal segments of 21 and 58 kbp, respectively (Fig. 1 and 4). In this report we have shown that there is a single, short viral DNA sequence, representing hardly more than 2,200 bp of virion DNA, which is abundantly transcribed in 1670 cells. Map posi-

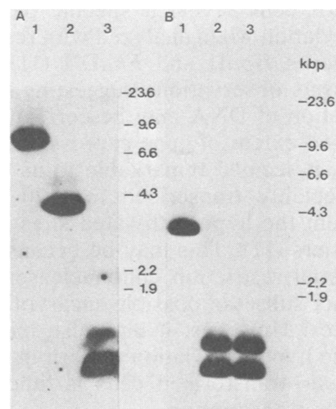

FIG. 5. Hybridization of ^{32}P-labeled cDNA from 1670 cells with cloned restriction fragments of *H. saimiri* L-DNA. (A) Oligo(dT)-primed cDNA hybridizing with the *Eco*RI C fragment cleaved by *Hpa*II (lane 1), with the *Eco*RI C fragment cleaved by *Kpn*I and *Hpa*II (lane 2), and with the *Kpn*I D fragment cleaved with *Hin*dIII (lane 3). (B) Randomly primed cDNA hybridizing with the *Eco*RI C fragment cleaved with *Kpn*I and *Hpa*II (lane 1), with the *Kpn*I D fragment cleaved by *Kpn*I, *Hpa*II, and *Hin*dIII (lane 2), and with the *Kpn*I D fragment cleaved by *Kpn*I and *Hin*dIII (lane 3).

tion and orientation of the coding sequence is shown in Fig. 4. A few experiments indicated that other parts of the viral genome may be expressed to a very low extent, if at all (data not shown).

Northern blot hybridizations, using rRNAs as size markers, seem to indicate that the predominant transcript of 1670 cells has 2.7 kb. On the other hand, hybridizations with randomly primed cDNA have clearly shown that the coding sequence is restricted essentially to two *Hin*dIII fragments of viral DNA (940 and 1,270 bp). This apparent discrepancy could be explained by the known inaccuracies of RNA size determination in denaturing agarose gels (25), or it may be due to the length of polyadenylic acid tails. It may also be possible that the transcribed viral DNA region in 1670 is amplified by short duplications which have been missed in the structural analysis of circular molecules performed thus far. The precise localization of the promoter and polyadenylation sites with respect to cloned virion DNA remains to be determined by analyses with single strand-specific DNAases. Sensitive Southern blot hybridizations with cloned probes did not give any indication that viral DNA may be integrated into the cellular genome (24) (S. Schirm and B. Fleckenstein, unpublished data); thus, it seems unlikely that the predominant RNA described here is a composite transcript of cellular and integrated viral DNA.

The transformed lymphoid cell line 1670 and fresh tumor cells reveal a specific pattern of hypomethylation when analyzed with restriction endonucleases *Hpa*II and *Fnu*DII (11). There are numerous observations suggesting that hypomethylation of DNA may be correlated with an increased extent of gene expression (13, 14, 32). Thus, it seemed remarkable to us that the DNA detectably transcribed in 1670 cells is remote from the hypomethylated sites mapped by Desrosiers (11). This may be because available bacterial restriction endonucleases recognize a minor subset of possible eucaryotic methylation sites. However, it may also mean that the specific hypomethylation pattern in L-DNA of *H. saimiri*-transformed cells is functionally unrelated to transcription.

The viral sequence transcribed in 1670 cells appears to correlate exactly with the segment of virion DNA that is predominantly transcribed during the immediate early phase of virus replication in the productive system (W. Bodemer, S. Angermüller, E. Knust, and B. Fleckenstein, unpublished data). It suggests that the active promoter in the circular DNA molecules of 1670 cells is independent of regulatory effects by other viral gene products. Circular viral DNA molecules in *H. saimiri*-transformed nonproducer cells generally have large deletions of L-DNA (24); however, the two short *Hin*dIII fragments that are transcribed in 1670 cells were always found to be conserved in all transformed cell lines analyzed so far (S. Schirm and B. Fleckenstein, unpublished data). This may indicate that the respective immediate early gene may be functionally related to the persistence of viral DNA in lymphoid cells or to the maintenance of transformation. This conclusion correlates well with our preliminary observation that the homologous viral DNA sequences are expressed in lymphoid cells transformed by *Herpesvirus ateles*. Despite appreciable transcription, no virus-specific protein could be detected in *H. saimiri*-transformed cells with conventional serological methods (23, 30). Sequence analysis of the two *Hin*dIII fragments transcribed in transformed cells may be the basis for future immunological studies with synthetic oligopeptides aimed at the detection of viral protein in *H. saimiri*-transformed cells.

Despite the similarities between Epstein-Barr virus-transformed lymphoblasts and *H. saimiri* cell lines, there are striking differences at the level of transcription. In *H. saimiri*-transformed cells, expression appears to be confined to a single immediate early gene. Epstein-Barr virus-transformed cells contain viral RNA of higher complexity, originating from at least three different regions of the viral genome (34, 39). In addition, Epstein-Barr virus-transformed lymphoblasts synthesize polymerase III transcripts in high abundancy (2, 26). The *H. saimiri*-transformed tumor line 1670 does not appear to produce analogous transcripts in detectable quantities.

ACKNOWLEDGMENTS

The work was supported by Deutsche Forschungsgemeinschaft (SFB 118).

The skillful technical assistance by Sieglinde Angermüller is greatly appreciated.

LITERATURE CITED

1. **Anderson, K. P., R. H. Costa, L. E. Holland, and E. K. Wagner.** 1980. Characterization of herpes simplex virus type 1 RNA present in the absence of de novo protein synthesis. J. Virol. **34:**9–27.
2. **Arrand, J. R., and L. Rymo.** 1982. Characterization of the major Epstein-Barr virus-specific RNA in Burkitt lymphoma-derived cells. J. Virol. **41:**376–389.
3. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidilic acid cellulose. Proc. Natl. Acad. Sci. U.S.A. **69:**1408–1412.
4. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18:**5294–5299.
5. **Clements, J. B., J. McLauchlan, and D. J. McGeoch.** 1979. Orientation of herpes simplex virus type 1 immediate-early mRNA's. Nucleic Acids Res. **7:**77–91.

6. **Colbère-Garapin, F., F. Horodniceanu, P. Kourilsky, and A.-C. Garapin.** 1981. A new dominant hybrid selective marker for higher eukaryotic cells. J. Mol. Biol. **150**:1–14.
7. **Deinhardt, F. W., L. A. Falk, and L. G. Wolfe.** 1974. Simian herpesviruses and neoplasia, p. 167–205. *In* G. Klein and S. Weinhouse (ed.), Advances in Cancer Research. Academic Press, Inc., New York.
8. **Denhardt, D. T.** 1966. A membrane-filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. **23**:641–646.
9. **Derman, E., K. Krauter, L. Walling, C. Weinberger, M. Ray, and J. E. Darnell, Jr.** 1981. Transcriptional control in the production of liver-specific mRNAs. Cell **23**:731–739.
10. **Desrosiers, R. C.** 1981. *Herpesvirus saimiri* DNA in tumor cells—deleted sequences and sequence rearrangements. J. Virol. **39**:497–509.
11. **Desrosiers, R. C.** 1982. Specifically unmethylated CG sites in *Herpesvirus saimiri* DNA in tumor cells. J. Virol. **43**:427–435.
12. **Desrosiers, R. C., C. Mulder, and B. Fleckenstein.** 1979. Methylation of *Herpesvirus saimiri* DNA in lymphoid tumor cell lines. Proc. Natl. Acad. Sci. U.S.A. **76**:3839–3843.
13. **Doerfler, W.** 1981. DNA-methylation—A regulatory signal in eukaryotic gene expression. J. Gen. Virol. **57**:1–20.
14. **Ehrlich, M., and R. Y.-H. Wang.** 1981. 5-methylcytosine in eukaryotic DNA. Science **212**:1351–1357.
15. **Falk, L. A., Jr.** 1980. Biology of *Herpesvirus saimiri* and *Herpesvirus ateles*, p. 813–832. *In* G. Klein (ed.), Viral oncology. Raven Press, New York.
16. **Fleckenstein, B.** 1979. Oncogenic herpesviruses of nonhuman primates. Biochim. Biophys. Acta **560**:301–342.
17. **Fleckenstein, B., G. W. Bornkamm, and H. Ludwig.** 1975. Repetitive sequences in complete and defective genomes of *Herpesvirus saimiri*. J. Virol. **15**:398–406.
18. **Fleckenstein, B., M. D. Daniel, R. D. Hunt, J. Werner, L. A. Falk, and C. Mulder.** 1978. Tumour induction with DNA of oncogenic primate herpesviruses. Nature (London) **274**:57–59.
19. **Fleckenstein, B., and R. C. Desrosiers.** 1982. *Herpesvirus saimiri* and *Herpesvirus ateles*, p. 253–332. *In* B. Roizman (ed.), The herpesviruses, vol. 1. Plenum Publishing Corp., New York.
20. **Fleckenstein, B., and C. Mulder.** 1980. p. 799–812. Molecular biological aspects of *Herpesvirus saimiri* and *Herpesvirus ateles*. *In* G. Klein (ed.), Viral oncology. Raven Press, New York.
21. **Fleckenstein, B., I. Müller, and J. Werner.** 1977. The presence of *Herpesvirus saimiri* genomes in virus-transformed cells. Int. J. Cancer **19**:546–554.
22. **Glisin, V., R. Crkvenjakov, and C. Byus.** 1974. Ribonucleic acid isolated by cesium chloride centrifugation. Biochemistry **13**:2633–2637.
23. **Johnson, D., S. Ohno, C. Kaschka-Dierich, B. Fleckenstein, and G. Klein.** 1981. Relationship between *Herpesvirus ateles*-associated nuclear antigen (HATNA) and the number of virus genome equivalents in HVA-carrying lymphoid lines. J. Gen. Virol. **52**:221–226.
24. **Kaschka-Dierich, C., F. J. Werner, I. Bauer, and B. Fleckenstein.** 1982. Structure of nonintegrated, circular *Herpesvirus saimiri* and *Herpesvirus ateles* genomes in tumor cell lines and in vitro-transformed cells. J. Virol. **44**:295–310.
25. **Lehrach, H., D. Diamond, J. M. Wozney, and H. Boedtker.** 1977. RNA molecular weight determination by gel electrophoresis under denaturating conditions, a critical reexamination. Biochemistry **16**:4743–4751.
26. **Lerner, M. R., N. C. Andrews, G. Miller, and J. A. Steitz.** 1981. Two small RNAs encoded by Epstein-Barr virus and complexed with protein are precipitated by antibodies from patients with systemic lupus erythematosus. Proc. Natl. Acad. Sci. U.S.A. **78**:805–809.
27. **Marczynska, B., L. Falk, L. Wolfe, and F. Deinhardt.** 1973. Transplantation and cytogenetic studies of *Herpesvirus saimiri*-induced phase in marmoset monkeys. J. Natl. Cancer Inst. **50**:331–337.
28. **McMaster, G. K., and G. C. Carmichael.** 1977. Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. Proc. Natl. Acad. Sci. U.S.A. **74**:4835–4838.
29. **Melendez, L. V., R. D. Hunt, M. D. Daniel, F. G. Garcia, and C. E. O. Fraser.** 1969. *Herpesvirus saimiri*. II. An experimentally induced malignant lymphoma in primates. Lab. Anim. Care **19**:378–386.
30. **Ohno, S., J. Luka, G. Klein, and M. D. Daniel.** 1979. Detection of a nuclear antigen in *Herpesvirus ateles*-carrying marmoset lines by the acid-fixed nuclear binding technique. Proc. Natl. Acad. Sci. U.S.A. **76**:2042–2046.
31. **Rabson, A. S., G. T. O'Conor, D. E. Lorenz, R. L. Kirschstein, F. Y. Legallais, and T. S. Tralka.** 1971. Lymphoid cell culture derived from lymph node of marmoset infected with *Herpesvirus saimiri*. Preliminary report. J. Natl. Cancer Inst. **46**:1099–1109.
32. **Razin, A., and A. D. Riggs.** 1980. DNA methylation and gene function. Science **210**:604–616.
33. **Rigby, P. N. J., M. Dieckmann, C. Rhoades, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity by nick-translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
34. **Rymo, L.** 1979. Identification of transcribed regions of Epstein-Barr virus DNA in Burkitt lymphoma-derived cells. J. Virol. **32**:8–18.
35. **Schildkraut, C. L., and S. Lifson.** 1965. Dependence of the melting temperature of DNA on salt concentration. Biopolymers **3**:195–208.
36. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
37. **Taylor, J. M., R. Illmensee, and J. Summers.** 1976. Efficient transcription of RNA into DNA by avian sarcoma virus polymerase. Biochim. Biophys. Acta **442**:324–330.
38. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. U.S.A. **77**:5201–5205.
39. **Van Santen, V., A. Cheung, and E. Kieff.** 1981. Epstein-Barr-virus RNA. VII. Size and direction of transcription of virus-specific cytoplasmic RNAs in a transformed cell line. Proc. Natl. Acad. Sci. U.S.A. **78**:1930–1934.
40. **Werner, F.-J., G. W. Bornkamm, and B. Fleckenstein.** 1977. Episomal viral DNA in a *Herpesvirus saimiri*-transformed lymphoid cell line. J. Virol. **22**:794–803.
41. **Werner, F.-J., R. C. Desrosiers, C. Mulder, G. W. Bornkamm, and B. Fleckenstein.** 1978. Physical mapping of viral episomes in *Herpesvirus saimiri* transformed lymphoid cells, p. 189–200. *In* J. G. Stevens, G. J. Todaro, and C. F. Fox (ed.), Persistent viruses. Academic Press, Inc., New York.