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Identification of transforming genes of subgroup A and C strains of *Herpesvirus saimiri*

(collagen-like repeat/focus formation/tumorigenesis)

JAE U. JUNG*, JOHN J. TRIMBLE*, NORVAL W. KING*, BRIGITTE BIESINGER†, BERNHARD W. FLECKENSTEIN†, AND RONALD C. DESROSIERS*‡

*New England Regional Primate Research Center, Harvard Medical School, Southborough, MA 01772; and †Institut fur Klinische and Molekulare Virologie der Universitat Erlangen-Nurnberg, D-8520 Erlangen, Federal Republic of Germany

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ABSTRACT **_Herpesvirus saimiri_ is an oncogenic herpesvirus that induces rapidly progressing lymphomas in New World primates. Using retrovirus vectors for gene transfer, specific open reading frames of _H. saimiri_ were tested for their ability to transform rodent cells in culture. One open reading frame, designated STP-C488 (for _saimiri_-transformation-associated protein of the subgroup C strain 488), phenotypically transformed Rat-1 cells, resulting in formation of foci, growth at reduced serum concentration, and growth to higher cell densities. Cells transformed by STP-C488 formed invasive tumors in nude mice. The STP-A11 reading frame of strain 11 (subgroup A) was much less potent in its transforming ability than STP-C488. These results demonstrate the oncogene nature of these two open reading frames and provide a means for studying their transforming functions independent of the rest of the _H. saimiri_ genome.**

Herpesvirus saimiri is a member of the gamma subfamily of herpesviruses (Gammaherpesvirinae). Some members of this lymphotropic group—e.g., Epstein–Barr virus, *H. saimiri*, *Herpesvirus ateles*, and *Herpesvirus sylvilagus*—are capable of inducing lymphoproliferative disorders in natural or experimental hosts. *H. saimiri* is apparently not associated with disease in its natural host, the squirrel monkey (*Saimiri sciureus*). However, it does induce rapidly progressing fatal lymphomas, leukemias, and lymphosarcomas in several other species of New World primates (for review, see ref. 1). Strains of *H. saimiri* have been divided into three subgroups (A, B, and C) based on the extent of DNA sequence divergence at the left end of L-DNA (2). Strains from subgroups A and C are highly oncogenic and are able to immortalize common marmoset peripheral blood lymphocytes *in vitro* to interleukin 2-independent growth (3).

Previous studies have shown that DNA sequences at the left terminus of L-DNA are required for *in vitro* immortalization and for the oncogenic phenotype (3, 4). Recent mutagenic analyses demonstrated that the left-most open reading frame (ORF) of the subgroup A strain 11 genome is required for immortalization of common marmoset T lymphocytes but not for replication of the virus (5). This ORF [*saimiri* transformation-associated protein (STP) of subgroup A strain 11 (STP-A11)] predicts a polypeptide with a highly hydrophobic stretch of amino acids at the carboxyl terminus, an acidic hydrophilic amino terminus, and a sequence appropriate for formation of a metal-binding domain (5). Two ORFs (ORF-1 and ORF-2) have been found in *H. saimiri* strain 488 (subgroup C) in a similar position and orientation in the viral genome as STP-A11 (6). Except for the hydrophobic, putative transmembrane domain at the carboxyl terminus, only limited sequence identities are apparent among STP-A11, ORF-1, and ORF-2. The translated sequence of ORF-2 of strain 488 contains a stretch of 54 amino acids (of 102 total) with collagen-like repeat motifs, mostly Gly-Pro-Pro. Close inspection of the STP-A11 sequence reveals that it, too, is proline rich, with scattered collagen-like repeats (six Gly-Xaa-Pro and three Gly-Pro-Xaa, where Xaas are other amino acids) (J.J.T., J.U.J., and R.C.D., unpublished data). However, the repetitive nature of the collagen-like repeat motifs in STP-A11 is nowhere near as striking as it is in ORF-2 of strain 488.

To investigate the role of these three ORFs, we examined their potential for transformation of rodent cells independent of the rest of the herpesviral genome. In this report we describe the ability of STP-A of strain 11 and ORF-2 of strain 488 to transform cells of the established rodent line Rat-1.

MATERIALS AND METHODS

Cell Culture. All cells were grown in Dulbecco's modified Eagle's medium (DMEM) with high glucose (4.5 g/liter) supplemented with 10% bovine calf serum (Hazelton, Lenexa, KS). An amphotropic retrovirus-packaging cell line GP+envAm-12 (a gift from A. Bank, Columbia University, New York) was selected in HXM medium containing hypoxanthine (15 μg/ml), xanthine (250 μg/ml), and mycophenolic acid (25 μg/ml) supplemented with hygromycin B (200 μg/ml). G418 (GIBCO) selection of drug-resistant cells after transfection or infection was done at a concentration of 500 μg/ml.

Plasmid Construction. DNA representing STP-A11 and ORF-2 was amplified from *H. saimiri* strains 11 and 488 by PCR. Oligonucleotides from complementary strands representing the 5′ and 3′ ends of the target genes were synthesized with *Eco*RI or *Bam*HI recognition sequences at their 5′ ends to facilitate cloning. STP-A11 includes base pairs (bp) 221–714 of the published sequence from the left end of L-DNA of *H. saimiri* strain 11 (5). ORF-2 includes bp 1051–1440 of the published sequence from the left end of L-DNA of *H. saimiri* strain 488 (6). The PCR cycling was accomplished with a DNA thermal cycler (Perkin–Elmer/Cetus) with the following conditions: 30 cycles of 2 min at 55°C for annealing, 2 min at 72°C for polymerization, and 1 min at 94°C for denaturation. The amplified DNA fragment containing STP-A11 or ORF-2 was ligated into *Eco*RI and/or *Bam*HI cloning site of the murine leukemia virus-based retroviral vector pLXSN (8). Orientation of the DNA insert in either the sense or antisense direction was determined by restriction endonuclease digestion. DNA inserts of the clones used were

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Abbreviations: STP, *saimiri*-transformation-associated protein; ORF, open reading frame; LTR, long terminal repeat.

‡To whom reprint requests should be addressed.

completely sequenced to verify 100% agreement with the original sequence.

A series of cloning steps was done to provide suitable ends for cloning of ORF-1 into the retroviral vector. A 887-bp *Spe* I–*Hin*cII DNA fragment containing ORF-1 was isolated from plasmid p488PX (6) and transferred into pBS KS+ vector (Stratagene) at *Spe* I and *Hin*cII sites. An *Xba* I–*Sal* I fragment containing ORF-1 DNA was transferred into pGM4Z vector and then an *Eco*RI–*Hin*dIII fragment from pGM4Z-ORF-1 containing ORF-1 was transferred again into pBS KS+ vector. Finally, an *Eco*RI–*Xho* I DNA insert containing ORF-1 was subcloned into *Eco*RI and *Xho* I cloning site of retroviral vector pLXSN. Orientation of the ORF-1 insert was confirmed by restriction endonuclease digestion and DNA sequencing.

Production of Recombinant Retrovirus. Vector DNA (10 μg) was introduced into the amphotropic packaging cell line GP+envAm-12 by electroporation (Bio-Rad) at 250 V and 960 μF in serum-free DMEM. After incubation for 48 hr with DMEM/10% serum, the transfected cells were cultured with selective medium containing G418 at 500 μg/ml for the next 3 weeks. To obtain the maximum viral titer, a minimal volume (0.1 ml/cm^2) of fresh culture medium was applied when the cells were 80–90% confluent and then left for 24 hr before harvesting the virus; the virus-containing supernatant was stored at −70°C. Virus supernatants were titered by infection of NIH 3T3 cells, as described (9). Rat-1 cells (5 × 10^5) were infected with medium containing recombinant retrovirus vectors from the packaging cell line, incubated for 2–4 hr, washed, and placed in fresh medium. G418 (500 μg/ml) was added to the medium after 48 hr, and cells were split when confluent.

Assays for Transformation Properties. For studies of serum dependence, 1 × 10^5 cells were seeded in 25-cm^2 flasks in DMEM/10% serum for 24 hr. The cultures were washed four times with serum-free medium and transferred to DMEM with 2, 1, 0.5, or 0% serum. The cells were observed daily, and medium was changed every 4 days for 2 weeks. In 0% serum condition, cells were trypsinized, and live cells were counted after trypan blue staining every 5 days.

Saturation densities were determined by counting cells in 25-cm^2 flasks at day 14 after plating 1 × 10^5 G418-resistant cells with the DMEM/10% serum changed every 4 days.

For assay of focus formation, 1 × 10^5 G418-resistant cells were plated in 100-mm tissue-culture dishes and maintained with DMEM/10% serum changed every 4 days. At day 14, cells were fixed for 3 hr in 10% formaldehyde, rinsed, stained with 0.1% methylene blue, and destained with distilled water. To measure the numbers of foci formed without previous selection of G418 resistance, 1 × 10^5 Rat-1 cells were transfected with 10 μg of constructed retroviral vector DNA by electroporation using the same conditions described above. After electroporation, cells were plated in 100-mm tissue-culture dishes and maintained with DMEM/10% serum changed every 4 days in the absence of G418 selection. The numbers of foci were determined as described above.

Tumorigenicity in Nude Mice. Fresh trypsinized cells (1 × 10^5 or 1 × 10^6) in 0.1 ml of DMEM without serum were injected s.c. into 5- to 6-week-old Swiss/athymic nude mice. Mice were observed for tumor development and euthanized to obtain tumor tissue samples.

Northern (RNA) Blotting. Total RNA from 1 × 10^7 G418-resistant cells or tumor tissues was prepared by the method of Chomczynski and Sacchi (10). Total RNA (20 μg) was fractionated by electrophoresis in agarose gels containing formaldehyde, transferred to nitrocellulose, and then subjected to Northern blot analysis. Retroviral vector DNA was labeled by nick-translation with [^{32}P]dNTP and was used for LXSN-specific probe (see Fig. 1). The gel-purified fragments containing STP-A11, ORF-1, or ORF-2 were nick-translated with [^{32}P]dNTP and also used for hybridization.

Pathology. The mice used in this study were euthanized by an overdose of ether anesthesia at 21 days after inoculation. Nodules present at the injection sites were dissected along with the overlying skin from the underlying tissues. Specimens were fixed in 10% buffered neutral formalin, embedded in paraffin, and processed for histopathologic evaluation using standard procedures. Each nodule was examined to determine its tumor cell morphology, pattern and extent of growth, mitotic activity, and other features that would distinguish one from another.

RESULTS

Construction of Retroviral Vectors. To assess the transforming potential of *H. saimiri* genes, we used a retroviral vector derived from murine leukemia virus for gene transfer and expression of three *H. saimiri* ORFs. The recombinant retroviral vector shown in Fig. 1 contains two transcriptional units: the 5′ Moloney murine sarcoma virus long terminal repeat (LTR) promoter and the internal simian virus 40 early promoter (8). To generate retrovirus vector-producing cell lines, 10 μg of plasmid DNA containing each retroviral construct was transfected into amphotropic packaging cell GP+envAM-12 by electroporation under the conditions described. After cells were placed in medium/G418 (500 μg/ml), only cells expressing the G418-resistance gene survived selection. The G418-resistant cells were amplified, and the culture medium was used as a source of virus. Virus-containing supernatant was analyzed for its ability to generate G418-resistant cell colonies by infection of NIH 3T3 cells. To obtain the maximum titer, a minimal volume (0.1 ml/cm^2) of fresh culture medium was applied when cells reached 80–90% confluence; the supernatant was then harvested 24 hr later and stored at −70°C. Virus titers of 1–2 × 10^6 colony-forming units per ml were obtained from all retrovirus vector constructs.

Expression of *H. Saimiri* Genes in Rat-1 Cells. The fibroblast cell line, Rat-1, was infected with LXSN retrovirus and recombinant retroviruses containing STP-A11, ORF-1, or ORF-2. G418-resistant Rat-1 cells were selected after each infection and called Rat–LXSN, Rat–STP-A11, Rat–ORF-1, and Rat–ORF-2, respectively. To demonstrate that introduced genes were transmitted correctly, the expression of *H. saimiri* genes in Rat-1 cells infected with the recombinant retroviruses was examined by Northern blot analysis of total cell RNA. Rat–LXSN cells contained a major RNA species of 3.0 kilobases (kb) that hybridized with labeled LXSN vector (Fig. 2) but not with *H. saimiri* sequences. Rat–STP-A11, Rat–ORF-1,

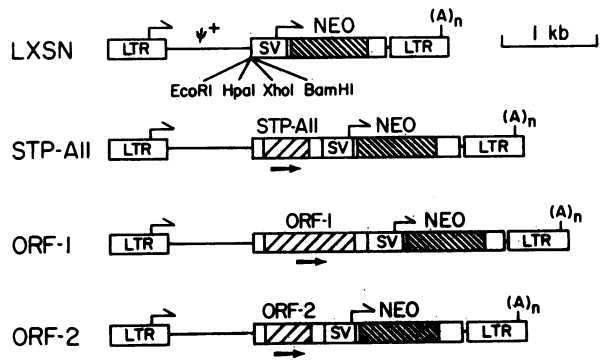

FIG. 1. Maps of recombinant retroviruses for transfer and expression of *H. saimiri* genes. Hatching indicates protein-coding region, and arrows indicate the initiation site and direction of transcription. (A)$_n$, poly(A) site; SV, simian virus 40 early promoter; NEO, neomycin phosphotransferase gene; Ψ$^+$, the retroviral packaging signal (8).

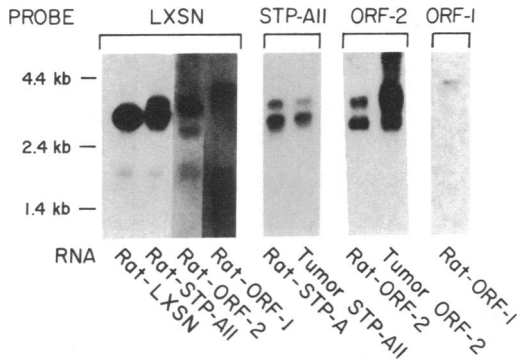

FIG. 2. Analysis of RNA from G418-resistant Rat-1 cells and tumor tissues. Total RNA isolations and hybridizations were done as described. RNA blots were hybridized with ^{32}P-labeled probes for LXSN, STP-A11, ORF-1, and ORF-2. Positions of RNA molecular size markers are indicated.

and Rat–ORF-2 cells contained major RNA species of 3.5, 4.0, and 3.4 kb that hybridized with both LXSN and *H. saimiri* probes. The sizes of these RNAs correspond to the expected sizes of the full-length proviral transcripts from the 5′ LTR of Moloney murine sarcoma virus. The different lengths of these RNAs reflect the sizes of the DNA fragments inserted into the vector. All cells also contained a 1.8-kb RNA species that hybridized with LXSN sequences but not with *H. saimiri* DNA, consistent with it being the neo^R transcript from the simian virus 40 promoter. Additionally, Rat–STP-A11 cells contained an RNA species of 3.0 kb, and Rat–ORF-2 cells contained one of 2.7 kb. It has been reported that the full-length mRNA is, by far, the most abundant in cells infected with LXSN retrovirus (8), but splicing from the normal Moloney murine sarcoma virus splice donor to cryptic splice acceptors just upstream of the cloning site can occur in cells infected with recombinant LXSN virus carrying inserted genes (11). The 3.0- and 2.7-kb viral transcripts were not seen in infected NIH 3T3 cells (data not shown).

Growth Characteristics of Rat-1 Cells Expressing *H. Saimiri* Genes. Growth properties of Rat–STP-A11 and Rat–ORF-2 cells differed markedly from those of the parent cells, Rat–LXSN cells, and Rat–ORF-1 cells. The expression of STP-A11 and ORF-2 stimulated Rat-1 cells to grow to higher cell densities (2- to 3-fold) than those of Rat–LXSN and Rat–ORF-1 cells (Table 1).

In focus-forming assays, Rat–ORF-2 cells formed many foci of deeply heaped up cells (Fig. 3, *3a* and *3b*). Foci were recognizable before cells reached confluence. Rat–STP-A11 cells also formed foci, but foci were fewer in number than in Rat–ORF-2 cells; the numbers of foci observed for Rat–ORF-2 and Rat–STP-A11 cells are >1000 and 300, respectively, per 1 × 10^5 G418-resistant cells with the conditions described (Fig. 3, *2a*, *2b*, and *3a*). The focus-forming assay from the transfection of ORF-2 DNA directly into Rat-1 cells without selection for G418 resistance showed an average number of 250 foci per 1 × 10^5 cells after 14 days, whereas the numbers of foci from transfection of STP-A11 DNA into Rat-1 cells without selection of G418 resistance were very few (<20 foci) per 1 × 10^5 cells (data not shown). By contrast, Rat–LXSN and Rat–ORF-1 cells grew into flat monolayers similar to normal Rat-1 cells and did not pile up (Fig. 3, *1a* and *1b*, *4a* and *4b*).

Table 1. Growth properties of Rat-1 cell lines expressing *H. saimiri* genes

Cell line	Saturation cell density* (×10^6 cell)	Growth in reduced serum concentration† 2%	1%	0.5%
Rat–LXSN	5.2	+	−	−
Rat–STP-A11	14.0	+	−	−
Rat–ORF-1	5.8	+	−	−
Rat–ORF-2	13.7	+	+	+

*Saturation cell densities were determined by counting cells in 25-cm^2 flasks at day 14 after plating 1 × 10^5 cells with medium changes every 4 days.

†For studies of serum dependence, 1 × 10^5 cells were seeded in 25-cm^2 flasks in DMEM/10% donor bovine calf serum for 24 hr and then washed four times with serum-free medium and transferred to DMEM with 2, 1, or 0.5% serum. +, Cells reached confluence with a >3-fold increase in cell number.

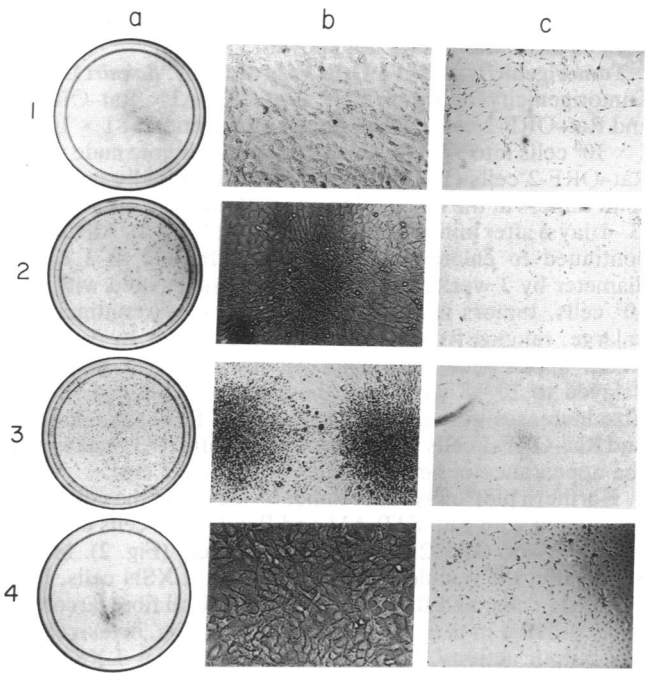

FIG. 3. Growth characteristics of Rat-1 cells expressing *H. saimiri* genes. (*1*) Rat–LXSN cells; (*2*) Rat–STP-A cells; (*3*) Rat–ORF-2 cells; (*4*) Rat–ORF-1 cells. G418-resistant cells were obtained after infection with recombinant retroviruses. G418-resistant cells were plated at 1 × 10^5 cells per 100-mm tissue-culture dishes, incubated 14 days, and stained with methylene blue to visualize foci (*a*) or photographed at ×50 to show morphologic transformation (*b*). For serum dependence (*c*), 1 × 10^5 G418-resistant cells were placed in 25-cm^2 flasks, maintained in 0% serum for 14 days, and photographed at ×25.

Serum dependence of the cell lines was tested over 14 days at 2, 1, 0.5, and 0% serum. At 1% and 0.5% serum concentrations, Rat–LXSN, Rat–STP-A11, and Rat–ORF-1 cells showed little, if any, increase in cell numbers (Table 1). In contrast, Rat–ORF-2 cells continued to grow, reached higher cell densities than Rat–LXSN, Rat–STP-A11, or Rat–ORF-1 cells, and still heaped up at 1% and 0.5% serum concentrations. However, it took five times longer in 1% and 0.5% serum for Rat–ORF-2 cells to reach a cell density close to that achieved in 10% serum. In 0% serum, Rat–LXSN, Rat–STP-A11, and Rat–ORF-1 cells changed from spindle to round and finally started to detach from the surface of the flask by 10 days (Fig. 3, *1c*, *2c*, and *4c*). Rat–ORF-2 cells were thinner in 0% serum than in 10% serum, but they maintained their morphology even in 0% serum for up to 30 days. In addition, they remained attached to the surface after 14 days in 0% serum (Fig. 3, *3c*). To determine survival in the absence of serum, Rat–LXSN and Rat–ORF-2 cells were incubated in 0% serum for 30 days. Although ORF-2 expression did not render Rat-1 cell growth independent of serum, Rat–ORF-2 cells remained attached for longer periods and contained

many more live cells as compared with Rat–LXSN after 30 days of incubation (data not shown).

Tumorigenicity of Rat-1 Cells Expressing *H. Saimiri* Genes. Tumorigenicity of Rat–LXSN, Rat–STP-A11, Rat–ORF-1, and Rat–ORF-2 cells was assessed by injection of 1×10^5 or 1×10^6 cells into 5- to 6-week-old Swiss/athymic nude mice. Rat–ORF-2 cells (1×10^6 cells) produced rapidly enlarging solid tumors at the inoculation site within a very short period (3–4 days) after injection into nude mice (Table 2). All tumors continued to enlarge rapidly, ranging from 2 to 3 cm in diameter by 3 weeks. When mice were inoculated with 1×10^5 cells, tumors appeared at 7–10 days and continued to enlarge, ranging from 0.5 to 1 cm in diameter by 3 weeks. Initial appearance of tumors by Rat–STP-A11 cells was delayed to 10–18 days, and these tumors enlarged slowly. Size increases near the injection sites of Rat-1, Rat–LXSN, and Rat–ORF-1 cells were not seen until after ≈3 weeks, with the appearance of small nodules (<0.2 × 0.2 cm).

Northern blot analyses of tumor tissues from the nude mice inoculated with Rat–STP-A11 and Rat–ORF-2 cells detected STP-A11 and ORF-2 mRNAs, respectively (Fig. 2). Microscopically, the nodules derived from Rat–LXSN cells, Rat–STP-A11 cells, and Rat–ORF-2 cells were all fibrosarcomas. They differed in their degree of differentiation, invasiveness, and mitotic activity. Those derived from the Rat–LXSN cells were the most differentiated and consisted of a uniform population of fibroblasts arranged in interlacing fasciculi separated by small amounts of collagen (Fig. 4, *1a* and *1b*). Mitotic figures were not common. The sarcomas derived from the Rat–STP-A11 cells were less differentiated and composed of cells with more pleomorphic and hyperchromatic nuclei and less cytoplasm. They were arranged in short interlacing fasciculi and whorls (Fig. 4, *2a* and *2b*). Mitotic figures were present in moderate numbers. The tumors derived from Rat–LXSN cells and Rat–STP-A11 cells were confined to the deeper portions of the subcutis. Fibrosarcomas derived from Rat–ORF-2 cells were the least differentiated and the most invasive. They were arranged in short, interlacing bundles that invaded the dermis subcutis and underlying skeletal musculature. Hair follicles and cutaneous adnexae were replaced by the neoplastic growth. Unlike those fibrosarcomas derived from the other two cell types, those derived from Rat–ORF-2 contained numerous mitotic figures and extensive areas of necrosis resulting from outgrowth of their blood supply (Fig. 4, *3a* and *3b*).

DISCUSSION

The results described here demonstrate that ORF-2 of strain 488 and STP-A of strain 11 of *H. saimiri* have transforming and tumor-inducing activities independent of the rest of the herpesviral genome. Specifically, ORF-2 can transform the established Rat-1 cell line to apparent loss of contact inhibition, formation of cellular foci, growth at reduced serum concentration, and tumorigenicity in nude mice, whereas STP-A11 can weakly transform the Rat-1 cells. We thus designate ORF-2 of strain 488 as STP-C488 for STP of the subgroup C strain 488.

Table 2. Tumorigenicity of Rat-1 cells in nude mice

Cell line	Cells, no.	Average tumor size (diameter), cm	
		Day 14	Day 21
Rat-1 (0/2)	1×10^6		DT
Rat–LXSN			
(0/2)	1×10^6		DT
(0/3)	1×10^5		
Rat–STP-A11			
(2/2)	1×10^6	0.2	0.3
(3/3)	1×10^5		0.2
Rat–ORF-1 (0/2)	1×10^6		DT
Rat–ORF-2			
(2/2)	1×10^6	2	3
(4/4)	1×10^5	0.7	1

Fresh trypsinized cells in 0.1 ml of DMEM without serum were injected s.c. into 5- to 6-week-old Swiss/athymic nude mice. Numbers in parentheses indicate tumor incidences per number of mice used. DT, diffuse trace: <0.2 cm.

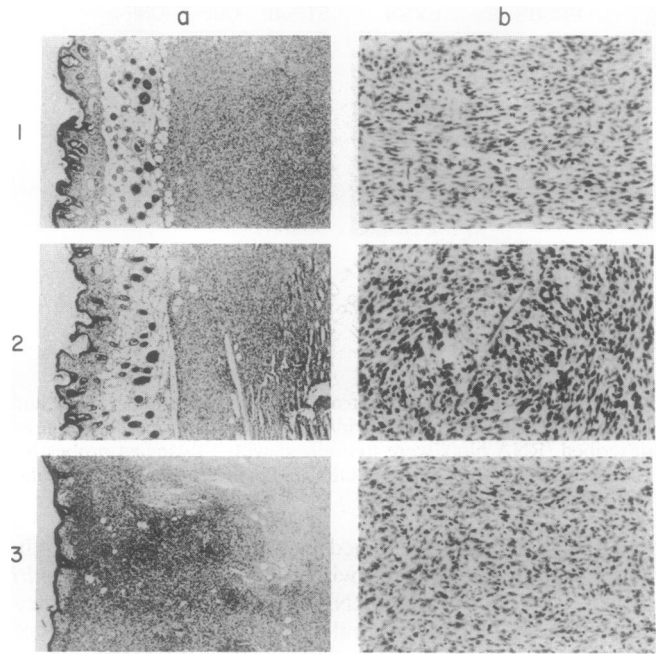

FIG. 4. Low- (*a*) and high- (*b*) magnification photomicrographs of tissue sections of skin and underlying fibrosarcomas from nude mice injected s.c. 21 days earlier with Rat–LXSN cells (*1*), Rat–STP-A11 cells (*2*), and Rat–ORF-2 cells (*3*). The sarcomas derived from Rat–LXSN cells (*1a*) and Rat–STP-A11 cells (*2a*) are confined to the deeper portions of the subcutis, whereas those derived from Rat–ORF-2 cells extend to the epidermis and have replaced hair follicles and adnexal structures. The pale area in the upper right portion of *3a* is an area of tumor necrosis. At higher magnification, the Rat–LXSN-derived tumor cells are fusiform and arranged in interlacing fasciculi typical of a fibrosarcoma (*1b*). The Rat–STP-A11-derived tumor cells are similar but have more hyperchromatic and pleomorphic nuclei and a higher nuclear–cytoplasmic ratio. They are arranged in short fasciculi and whorls (*2b*). The Rat–ORF-2-derived tumor cells are pleomorphic, have a higher mitotic index, and have undergone considerable necrosis, as evidenced by the amount of nuclear debris present (*3b*).

A similar ability to transform rodent cell lines has been demonstrated for *BARF1* and *LMP-1* genes of Epstein–Barr virus, polyoma virus middle tumor antigen, *tax* gene of human T-cell lymphotropic virus type 1, adenovirus *E1B*, *src*, *myc*, tyrosine kinase oncogenes, and *ras*, among others (12–14). However, amino acid and DNA sequences of STP-A11 and STP-C488 show no identity to any of these transforming genes; nor do they contain any clear transformation-relevant sequence motifs or conserved sequences previously defined in other transforming genes. These sites include GTP-binding sites or transcriptional regulator-binding sites (15, 16). *H. saimiri* STP-A11 and STP-C488 thus appear to be unique transforming genes.

Although STP-A11 and STP-C488 share a similar location and orientation within the herpesviral genome of their respective strains, STP-A11 and STP-C488 display only limited sequence similarity (6). Nonetheless, STP-A11 and STP-C488 seem to be organized in a similar fashion in terms of the presence and location of specific structural motifs (Fig. 5). Both proteins have highly acidic amino termini. The pI of the

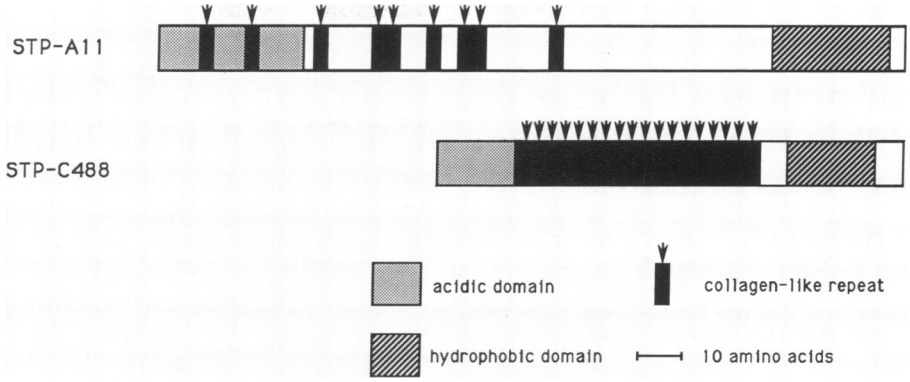

FIG. 5. Schematic diagram of the structural organization of STP-A11 and STP-C488. Collagen-like repeat is Gly-Xaa-Yaa, where Xaa and/or Yaa is proline.

amino terminal 32 amino acids of STP-A11 was calculated to be 3.5. The amino terminal 17 amino acids of STP-C488, up to but not including the collagen repeats, had a calculated pI of 4.2. The primary amino acid sequence of STP-A11 has nine collagen-like motifs (Gly-X-Y, where X and/or Y is proline), which is directly repeated 18 times in STP-C488 (ref. 6; J.J.T., J.U.J., and R.C.D., unpublished data). The collagen-like motifs in STP-A11 are not directly repeated, as they are in STP-C488, but they are similarly concentrated in the central portion of the protein. We do not as yet know the biochemical activities of the products of these collagen-like ORFs in *H. saimiri*. It has been found that collagen has a function in growth regulation (17), and small collagen-like polypeptides also have stimulating effects on embryonic tissues (18) and bone-marrow stem cell cultures (19). Several proteins including the type 1 macrophage scavenger receptor (20), rat mannose-binding protein (21), human pulmonary surfactant apoprotein (22), and human complement c1q subcomplement B chain and C chain (23, 24) have also been found to contain collagen-like repeats. In most cases, these collagen-like regions appear to have a structural rather than a functional role. However, it will be interesting to learn the roles of these collagen-like motifs in the transforming activities of STP-A11 and STP-C488.

STP-A11 and STP-C488 also share a hydrophobic, putative transmembrane domain at their carboxyl termini (Fig. 5). Analysis of DNA sequences from six subgroup A isolates of *H. saimiri* has demonstrated that the hydrophobic carboxyl terminus is highly conserved in STP-A (J.J.T., J.U.J., and R.C.D., unpublished data). Recently published DNA sequences from the same region of another subgroup C strain (484) also revealed two ORFs at this end of the genome (25). One of these ORFs in strain 484 was reported to contain collagen-like repeat motifs similar to STP-C488, but its carboxyl terminus was not hydrophobic (25). However, we recently sequenced this region of the 484 genome and found four errors in the published sequence of Geck *et al.* (25). The corrected sequence reveals a hydrophobic stretch at the carboxyl terminus of this ORF in strain 484 with an amino acid sequence very similar to STP-C488. Thus, STP-A11, STP-C488, and the corresponding ORF in the group C strain 484 all contain a hydrophobic stretch of amino acids at their carboxyl termini.

Mutational analyses have shown the requirement of STP-A for immortalization of marmoset lymphocytes *in vitro* and for oncogenicity *in vivo* by group A strains of *H. saimiri* (5). Analysis of recombinants between subgroup B and C strains has similarly indicated a role for corresponding sequences in subgroup C strains in oncogenic transformation (7). The results described in this report demonstrate directly the transforming properties of STP genes, and they provide a means for studying their transforming functions independent of the rest of the *H. saimiri* genome.

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1. Fleckenstein, B. & Desrosiers, R. C. (1982) in *The Herpesviruses*, ed. Roizman, B. (Plenum, New York), Vol. 1, pp. 253–332.
2. Medveczky, P., Szomolanyi, E., Desrosiers, R. C. & Mulder, C. (1984) *J. Virol.* **52,** 938–944.
3. Desrosiers, R. C., Silva, D. P., Waldron, L. M. & Letvin, N. L. (1986) *J. Virol.* **57,** 701–705.
4. Desrosiers, R. C., Bakker, A., Kamine, J., Falk, L. A., Hunt, R. D. & King, N. W. (1985) *Science* **228,** 184–187.
5. Murthy, S. C. S., Trimble, J. J. & Desrosiers, R. C. (1989) *J. Virol.* **63,** 3307–3314.
6. Biesinger, B., Trimble, J. J., Desrosiers, R. C. & Fleckenstein, B. (1990) *Virology* **176,** 505–514.
7. Medveczky, M. M., Szomolanyi, E., Hesselton, R., DeGrand, D., Geck, P. & Medveczky, P. G. (1989) *J. Virol.* **63,** 3601–3611.
8. Miller, A. D. & Rosman, G. J. (1990) *BioTech.* **7,** 980–990.
9. Compere, S. J., Baldacci, P. A., Sharpe, A. H. & Jaenisch, R. (1989) *Mol. Cell. Biol.* **9,** 6–14.
10. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
11. Hock, R. A., Miller, A. D. & Osborne, W. R. A. (1989) *Blood* **74,** 876–881.
12. Bishop, M. J. (1985) *Cell* **42,** 23–38.
13. Wang, D., Liebowitz, D. & Kieff, E. (1985) *Cell* **43,** 831–840.
14. Wei, M. X. & Ooka, T. (1989) *EMBO J.* **8,** 2897–2903.
15. Bourne, H. R., Sanders, D. A. & McCormick, F. (1991) *Nature (London)* **349,** 117–127.
16. Landschulz, W. H., Johnson, P. F. & McKnight, S. L. (1988) *Science* **240,** 1759–1764.
17. Nugent, M. A. & Newman, M. J. (1989) *J. Biol. Chem.* **264,** 18060–18067.
18. Sage, H. (1985) *Biochemistry* **24,** 7430–7440.
19. Waterhouse, E. J., Quisenberry, P. J. & Balian, G. (1986) *J. Cell. Physiol.* **127,** 397–402.
20. Kodama, T., Freeman, M., Rohrer, L., Zabrecky, J., Matsudaira, P. & Krieger, M. (1990) *Nature (London)* **343,** 531–535.
21. Drickamer, K., Dordal, M. S. & Reynolds, L. (1986) *J. Biol. Chem.* **261,** 6878–6887.
22. White, R. T., Damm, D., Miller, J., Spratt, K., Schilling, J., Hawgood, S., Benson, B. & Cordell, B. (1985) *Nature (London)* **317,** 361–363.
23. Reid, K. B. M. (1979) *Biochem. J.* **179,** 367–371.
24. Reid, K. B. M. (1985) *Biochem. J.* **231,** 729–735.
25. Geck, P., Whitaker, S. A., Medveczky, M. M. & Medveczky, P. G. (1990) *J. Virol.* **64,** 3509–3515.