

Retrovirus Restriction by TRIM5 α Variants from Old World and New World Primates

Byeongwoon Song,¹ Hassan Javanbakht,¹ Michel Perron,¹ Do Hyun Park,¹ Matthew Stremlau,¹ and Joseph Sodroski^{1,2*}

Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Department of Pathology, Division of AIDS, Harvard Medical School,¹ and Department of Immunology and Infectious Diseases, Harvard School of Public Health,² Boston, Massachusetts

Received 9 September 2004/Accepted 3 November 2004

The TRIM5 α proteins of humans and some Old World monkeys have been shown to block infection of particular retroviruses following virus entry into the host cell. Infection of most New World monkey cells by the simian immunodeficiency virus of macaques (SIV_{mac}) is restricted at a similar point. Here we examine the antiretroviral activity of TRIM5 α orthologs from humans, apes, Old World monkeys, and New World monkeys. Chimpanzee and orangutan TRIM5 α proteins functionally resembled human TRIM5 α , potently restricting infection by N-tropic murine leukemia virus (N-MLV) and moderately restricting human immunodeficiency virus type 1 (HIV-1) infection. Notably, TRIM5 α proteins from several New World monkey species restricted infection by SIV_{mac} and the SIV of African green monkeys, SIV_{agm}. Spider monkey TRIM5 α , which has an expanded B30.2 domain v3 region due to a tandem triplication, potently blocked infection by a range of retroviruses, including SIV_{mac}, SIV_{agm}, HIV-1, and N-MLV. Tandem duplications in the TRIM5 α B30.2 domain v1 region of African green monkeys are also associated with broader antiretroviral activity. Thus, variation in TRIM5 α proteins among primate species accounts for the observed patterns of postentry restrictions in cells from these animals. The TRIM5 α proteins of some monkey species exhibit dramatic lengthening of particular B30.2 variable regions and an expanded range of susceptible retroviruses.

Following entry into the host cell, retroviruses must negotiate a series of processes to establish a permanent infection of the host cell. These include the uncoating of the viral core, reverse transcription, nuclear access, and integration of the viral DNA into the host genome (1, 45). Early, postentry restrictions to retrovirus infection can determine tropism at the species level (6, 14, 15, 32, 36). Infection by N-tropic murine leukemia virus (N-MLV), for example, is inefficient in most human cells and certain cell lines from African green monkeys (4, 39). Human immunodeficiency virus type 1 (HIV-1) encounters a postentry block in Old World monkeys, whereas simian immunodeficiency virus of macaques (SIV_{mac}) is blocked in most New World monkey cells (14, 15, 32). These species-specific restrictions share several features. First, the block occurs prior to or concurrent with reverse transcription, which occurs in the cytoplasm of the host cell. At most, low levels of early reverse transcripts are made in restricted cells (7, 14, 23, 32). Second, the viral determinant of susceptibility to the block is the capsid protein (7, 11, 19, 25, 26, 39). Other capsid-binding proteins, such as cyclophilin A in the case of HIV-1, can modify the degree of the restriction (25, 27, 41). Third, the restriction is mediated by dominant host factors, the activity of which can be titrated by the introduction of virus-like particles containing proteolytically processed capsid proteins of the restricted viruses (3, 7, 10, 23, 25, 40).

These observations suggested a model in which host restric-

tion factors interact, directly or indirectly, with the viral capsid and prevent its progression along the infection pathway. A tripartite motif (TRIM) protein, TRIM5 α , was identified as the major factor in rhesus monkey cells restricting HIV-1 infection (37). The rhesus monkey TRIM5 α (TRIM5 α_{rh}) was shown to confer potent resistance to HIV-1 infection in otherwise susceptible cells and was necessary for the maintenance of the block to the early phase of HIV-1 infection in Old World monkey cells (37). Cells expressing TRIM5 α_{rh} exhibited partial inhibition of SIV_{mac} infection but were as susceptible as control cells to infection by Moloney murine leukemia virus (MoMLV) vectors (37). Humans express a protein, TRIM5 α_{hu} , that is 87% identical in amino acid sequence to the rhesus monkey protein, TRIM5 α_{rh} . Even when expressed at comparable levels, TRIM5 α_{hu} was less potent at suppressing HIV-1 and SIV_{mac} infection than TRIM5 α_{rh} (12, 37, 47). Recently, TRIM5 α_{hu} was shown to be responsible for the postentry restriction of N-MLV in human cells (12, 17, 27, 47). TRIM5 α_{rh} was less effective than TRIM5 α_{hu} at blocking these murine leukemia viruses (27). The TRIM5 α protein from African green monkeys, TRIM5 α_{agm} , inhibited N-MLV, HIV-1, and SIV_{mac} infection (12, 17, 47). These observations underscore the importance of species-specific variation in TRIM5 orthologs to the ability to restrict infection by particular retroviruses.

The tripartite motif that defines the TRIM proteins includes a RING domain, B-box 2 domain, and coiled-coil domain (28). Some TRIM proteins, including TRIM5, contain a C-terminal B30.2 or SPRY domain (13, 28). Differential splicing of the TRIM5 primary transcript gives rise to the expression of several isoforms of the protein product (28). The TRIM5 α isoform is the largest product (493 amino acid residues in hu-

* Corresponding author. Mailing address: Dana-Farber Cancer Institute, 44 Binney St., JFB 824, Boston, MA 02115. Phone: (617) 632-3371. Fax: (617) 632-4338. E-mail: joseph_sodroski@dfci.harvard.edu.

mans) and contains the B30.2/SPRY domain. The other TRIM5 isoforms (γ and δ are the best substantiated of these) lack an intact B30.2/SPRY domain. The TRIM5 γ_{rh} isoform does not inhibit HIV-1 or SIV $_{mac}$ infection (37). Thus, the B30.2 domain is critical for the ability of TRIM5 α to mediate antiretroviral effects.

Studies of endogenous retroviral sequences indicate that vertebrates, including humans, have been exposed to retroviruses for many millions of years (2, 16, 18, 20–22, 34, 35, 42–44, 46). This long history of host-retrovirus coevolution might favor the selection of particular TRIM5 α proteins that effectively suppress lethal retrovirus infections. Conversely, retroviral capsids may have evolved to minimize the impact of the TRIM5 α protein produced by the favored host species. Here we clone the TRIM5 cDNAs of several primate species and study the antiretroviral activities of these TRIM5 α variants.

MATERIALS AND METHODS

Cells for TRIM5 cDNA synthesis. SQMK-FP (Pindak) cells from a Bolivian squirrel monkey (*Saimiri boliviensis boliviensis*), Vero cells from an African green monkey (*Cercopithecus aethiops pygerythrus*), COS-1 cells from another subspecies (*Cercopithecus aethiops tantalus*) of African green monkey, murine NIH3T3 cells, and human HeLa cells were obtained from the American Type Culture Collection. Primary rhesus lung fibroblasts were derived from a rhesus macaque (*Macaca mulatta*) (15). The Coriell Institute for Medical Research (Camden, N.J.) supplied the following cells: AG05352 from a black-handed spider monkey (*Ateles geoffroyi*), AG05308 from a red-chested mustached tamarin (*Saguinus labiatus*), AG06209 from a Sumatran orangutan (*Pongo pygmaeus abelii*), and GM03448 from a chimpanzee (*Pan troglodytes verus*).

TRIM5 cDNA cloning and sequencing. First- and second-strand cDNA synthesis employed a cDNA synthesis kit (Clontech), using RNA prepared from the cells described above. Human and primate TRIM5 cDNAs encoding TRIM5 α were amplified using TRIMf2, 5'-GCGGAATTCGCCATGGCTTCTGGAATCCTGGTT-3', and TRIMr2, 5'-GCGATCGATGCCTCAAGAGCTTGGTGAGCAG-3'.

Amplification was carried out using the Clontech Advantage PCR kit, thermocycling the reactions at 95°C for 30 s followed by 30 cycles of 95°C for 30 s, 55°C for 1 min, and 68°C for 3 min. Amplified cDNAs were inserted into the pCR-BluntII-TOPO plasmid (Invitrogen) and sequenced using the following primers: M13f (-20), GTAAAACGACGGCCAGT; M13r (-21), AACAGCTATGACCATG; TRIMf3, GGAAGCTGACATCAGAGA; TRIMf4, GATAAGAGACAAGTGAGC; TRIMr3, TCTACCTCCAGTAATG; and TRIMr4, TCCTCTCAGGTTTTCG.

Creation of cells stably expressing TRIM5 α variants. Retroviral vectors encoding the TRIM5 α variants were created by using the pLPCX vector (37). The pLPCX-TRIM5 α vectors contain only the amino acid-coding sequence of the TRIM5 α cDNA. With the exception of human TRIM5 α , the TRIM5 α proteins possess N-terminal epitope tags derived from influenza virus hemagglutinin (HA). In the human TRIM5 α construct, the HA tag is at the C terminus (37). Recombinant viruses were produced in 293T cells by cotransfecting these pLPCX plasmids with the pVPack-GP and pVPack-VSV-G packaging plasmids (Stratagene). The pVPack-VSV-G plasmid encodes the vesicular stomatitis virus (VSV) G envelope glycoprotein, which allows efficient entry into a wide range of vertebrate cells (5, 15, 48). The resulting virus particles were used to transduce $\sim 1 \times 10^5$ HeLa and NIH3T3 cells in the presence of 5 μ g of Polybrene/ml. Cells were selected in 1 μ g of puromycin (Sigma)/ml.

Immunoblotting. HA-tagged TRIM5 α proteins expressed stably in HeLa cells were detected by Western blotting of whole-cell lysates. Cells were lysed in 150 mM NaCl, 50 mM Tris-Cl (pH 7.5), and 1% Triton X-100. The primary antibody for detection of the HA tag was HA.11 (Covance, Berkeley, Calif.). As a control, β -actin was detected with the C-2 antibody (Santa Cruz Biotechnology). Following the binding of primary antibody, the membrane was incubated with horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology) and ECL reagent (Amersham).

Infection with viruses expressing GFP. Recombinant viruses (HIV-1-GFP, SIV $_{mac}$ -GFP, N-MLV-GFP, B-MLV-GFP, and MoMLV-GFP) expressing the humanized *Renilla reniformis* green fluorescent protein (GFP) were prepared as previously described (27, 37). The SIV $_{agm}$ -GFP virus was created from the

pSIV $_{agm}$ Tan-1 plasmid (33), which was provided by Marcelo Soares and Beatrice Hahn through the National Institutes of Health AIDS Research and Reference Reagent Program. A DNA fragment encoding GFP (Clontech) was inserted into XhoI and NotI sites in place of *nef* sequences 8900 to 9000 in the SIV $_{agm}$ Tan-1 provirus. A deletion from nucleotides 6300 to 7100 was introduced into an *env* subclone from this provirus, and the Age I-XhoI fragment containing the *env* deletion was cloned back into the proviral plasmid. Recombinant viruses were produced by transfection of 293T cells with this plasmid, pHCMV-G, and a Rev-expressing plasmid, as described elsewhere (15, 27, 37). HIV-1-GFP, SIV $_{mac}$ -GFP, and SIV $_{agm}$ -GFP viral stocks were quantified by measuring reverse transcriptase activity, as described previously (29). The infectivity of the N-MLV-GFP, B-MLV-GFP, and MoMLV-GFP viruses was determined by titrating the virus stocks on 293T cells. For infection, 3×10^4 HeLa or NIH 3T3 cells expressing the TRIM5 α variants were seeded in 24-well plates and incubated with 2×10^4 to 4×10^4 cpm of HIV-1-GFP or SIV-GFP viruses, or MLV-GFP viruses at a multiplicity of infection of 0.5, for 12 to 16 h. Cells were washed and returned to culture for 48 h and then subjected to fluorescence-activated cell sorter (FACS) analysis with a FACScan (Becton Dickinson).

Nucleotide sequence accession numbers. The primate TRIM5 nucleotide sequences have been deposited in the GenBank database under accession numbers AY740612 to AY740618.

RESULTS

TRIM5 α proteins from different primate species. To investigate the functional importance of variation in the TRIM5 α protein, TRIM5 cDNA was cloned from the cells of several primate species. In addition to the previously reported (12, 17, 37, 47) TRIM5 cDNAs from humans and Old World monkeys (rhesus monkeys and African green monkeys), TRIM5 cDNA was cloned from two ape species (chimpanzee and orangutan) and three New World monkeys (squirrel monkey, tamarin, and spider monkey). The predicted amino acid sequences of these primate TRIM5 α orthologs are aligned in Fig. 1. We note a few features of the primate TRIM5 α sequences potentially relevant to the function of these proteins. Key structural elements comprising the known TRIM domains, such as the cysteines and histidines of the RING and B-box 2 domains, are invariant among the primate TRIM5 α proteins studied. The sequences of the RING, B-box 2, and coiled-coil domains align well, with lineage-specific variation in individual amino acid residues apparent (Fig. 1). In the B30.2 domain, interspecies variation in TRIM5 α is dramatic. Sequence comparisons of the B30.2 domains of multiple TRIM proteins have revealed four variable regions, designated v1 to v4; in these variable regions, significant length polymorphism as well as individual residue variation occur (33a). The boundaries of v1, v2, and v3 are depicted in Fig. 1; the v4 region of primate TRIM5 α proteins exhibits no length polymorphism (33a) and is not shown. Dramatic length polymorphism is observed in the v1 and v3 regions of primate TRIM5 α proteins. Old World primate TRIM5 α proteins possess relatively long v1 regions, whereas New World primate TRIM5 α proteins exhibit relatively long v3 regions (33a). Of particular note, the TRIM5 α proteins of African green monkeys and related monkeys possess unusually long v1 regions as a result of tandem sequence duplications (Fig. 1). Similarly, the extremely long v3 region of spider monkeys, a New World species, arises from a tandem triplication. In summary, lineage-specific variation characterizes primate TRIM5 α proteins, particularly with respect to the B30.2 domains.

Expression of the primate TRIM5 α proteins. HeLa cells stably expressing the TRIM5 α proteins of different primates were established. All of the TRIM5 α proteins were engineered to contain an N-terminal epitope tag, except human TRIM5 α ,

	----- RING -----						
human	MASGILVNVK	EEVTCPCICLE	LLTQPLSLDC	GHSFCQACLT	ANHKKSM LDK	.GESSCPVCR	60
chimpanzee	MASGILVNVK	EEVTCPCICLE	LLTQPLSLDC	GHSFCQACLT	ANHKKSM LDK	.GESSCPVCR	
orangutan	MASGILVNVK	EEVTCPCICLE	LLTQPLSLDC	GHSFCQACLT	ANHKKSTL DK	.GERSCPVCR	
agm (pyg)	MASGILVNVK	EEVTCPCICLE	LLTEPLSLPC	GHS L CQACIT	ANHKESML YK	<u>EEERS</u> CPVCR	
agm (tan)	MASGILVNVK	EEVTCPCICLE	LLTEPLSLPC	GHSFCQACIT	ANHKESML YK	<u>EEERS</u> CPVCR	
rhesus m.	MASGIL LNVK	EEVTCPCICLE	LLTEPLSLHC	GHSFCQACIT	ANH K SML YK	<u>EGERS</u> CPVCR	
squirrel m.	MASGILV SIK	EEVTCPCICLE	LLTEPLSLDC	GHSFCQACIT	ANH KESML H	<u>QGERS</u> CP LCR	
tamarin	MASGILVNIK	EEVTCPCICLE	LLTEPLSLDC	GHSFCQACIT	ANHKESTPH	<u>QGERS</u> CP LCR	
spider m.	MASGIL LNIK	EEVTCPCICLE	LLTEPLSLDC	GHSFCQACIT	ANHKESTLH	<u>QGERS</u> CP LCR	
	----- B-Box 2 -----						
human	ISYQPENIRP	NRHVANLVEK	LREVKLSP.E	GQKVDH CARH	GEKLLLF CQE	DGKVICWLCE	120
chimpanzee	ISYQPENIRP	NRH AANIVEK	LREVKLSP.E	GQKVDH CAH	GEKLLLF CQE	DGKVICWLCE	
orangutan	VSYQPKNIRP	NRHVANIVEK	LREVKLSP.E	GQKVDH CARH	GEKLLLF CQE	DGKVICWLCE	
agm (pyg)	ISYQPENIQP	NRHVANIVEK	LREVKLSPEE	GQKVDH CARH	GEKLLLF CQE	<u>DSKVI</u> CWLCE	
agm (tan)	ISYQPENIQP	NRHVANIVEK	LREVKLSPEE	GQKVDH CARH	GEKLLLF CQE	<u>DSKVI</u> CWLCE	
rhesus m.	ISYQPENIQP	NRHVANIVEK	LREVKLSPEE	GQKVDH CARH	GEKLLLF CQE	<u>DSKVI</u> CWLCE	
squirrel m.	<u>LPYQSEN</u> L RP	NRH L ASIVER	LREVM L RPEE	<u>RQNV</u> DH CARH	GEKLLLF CQE	<u>DGNI</u> I CWLCE	
tamarin	<u>MSYPSEN</u> L RP	NRH LANIVER	LKEVM L SPEE	GQKVGH CARH	GEKLLLF CQE	<u>DGNVI</u> CWLCE	
spider m.	<u>VSYQSEN</u> L RP	NRH LANIAER	LREVM L SPKE	GQKVD R CARH	GEKLLLF CQ Q	<u>HGNVI</u> CWLCE	
	----- Coiled coil -----						
human	RSQEHRGHHT	F P TEEVAQ EY	QVKLQA ALEM	LRQKQQA EAE	LEADIRE EKA	SWKTQIQYDK	180
chimpanzee	RSQEHRGHHT	FL TEEVA R EY	QVKLQA ALEM	LRQKQQA EAE	LEADIRE EKA	SWKTQIQYDK	
orangutan	RSQEHRGHHT	FL TEEVA QKY	QVKLQA ALEM	LRQKQQA EAE	LEADIRE EKA	SWKTQIQYDK	
agm (pyg)	RSQEHRGHHT	FL MEEVAQ EY	<u>HVKLQ</u> TALEM	LRQKQQA EK	LEADIRE EKA	SWKI Q I D YDK	
agm (tan)	RSQEHRGHHT	FL MEEVAQ EY	<u>HVKLQ</u> TALEM	LRQKQQA EK	LEAD T REEKA	SWKI Q I D YDK	
rhesus m.	RSQEHRGHHT	FL MEEVAQ EY	<u>HVKLQ</u> TALEM	LRQKQQA EK	LEADIRE EKA	SWKI Q I D YDK	
squirrel m.	RSQEHRGHHT	FL VEEVA QKY	<u>REKLQ</u> VALET	<u>MRQKQ</u> DAEK	LEAD V R EQA	SWKI Q I R NDK	
tamarin	RSQEHRGHHT	LL VEEVA EK Y	<u>QEKLQ</u> VALEM	<u>MRQKQ</u> DAEK	LEAD V R EQA	SWKI Q I R NDK	
spider m.	RSQEHRGHST	FL VEEVA QKY	<u>QEKLQ</u> VALEM	<u>MRQKQ</u> DAEK	LE V D V R EQA	SWKI Q I E NDK	

human	TNVLADFEQL	RDILDWEESN	ELQNLEKEEE	DILKSLT NSE	TEMVQQTQSL	RELISDLEHR	240
chimpanzee	TNVLADFEQL	RDILDWEESN	ELQNLEKEEE	DILKSLTKSE	TEMVQQTQSV	RELISDLERR	
orangutan	TSVLADFEQL	RDILDWEESN	ELQNLEKEEE	DILKSLTKSE	TEMVQQTQSV	RELISDV EHR	
agm (pyg)	TNVSADFEQL	<u>RE</u> ILDWEESN	ELQNLEKEEE	DILKSLTKSE	TEMVQQTQVM	RELISDLEHR	
agm (tan)	TNVSADFEQL	<u>RE</u> ILDWEESN	ELQNLEKEEE	DILKSLTKSE	TEMVQQTQVM	RELISDLEHR	
rhesus m.	TNVSADFEQL	<u>RE</u> ILDWEESN	ELQNLEKEEE	DILKSLTKSE	TEMVQQTQVM	RELIS D LEHR	
squirrel m.	<u>TNIMAE</u> FKQL	RDILDCEESN	ELQNLEKEEK	<u>NILKRL</u> VQSE	<u>NDMVL</u> QTQSV	<u>RVLISD</u> LERR	
tamarin	<u>TNIMAE</u> FKQL	RDILDCEESK	ELQNLEKEEK	<u>NILKRL</u> VQSE	<u>SDMVL</u> QTQSM	<u>RVLISD</u> LERR	
spider m.	<u>TNILAE</u> FKQL	RDILDCEESN	ELQNLEKEEE	<u>NLLKTL</u> AQSE	<u>NDMVL</u> QTQSM	<u>RVLIA</u> DLEHR	

human	LQGSVMELLQ	GVDGVIK RTE	NVTLKKPETF	PKNQRRVFRA	PDLKGM L EVF	RELTDVRRYW	300
chimpanzee	LQGSVMELLQ	GVDGVIK RME	NVTLKKPETF	PKNQRRVFRA	PDLKGM L EVF	RELTDVRRYW	
orangutan	LQGSVMELLQ	GVDG I IKRMQ	NVTLKKPETF	PKNQRRVFRA	<u>PNLKGM</u> L EVF	RELTDVRRYW	
agm (pyg)	LQGS MELLQ	GVDG I IKRVE	NMTLKKPKTF	HKNQRRVFRA	PDLKGM L DMF	RELTDVRRYW	
agm (tan)	LQGS MELLQ	GVDG I IKRIE	NMTLKKPKTF	HKNQRRVFRA	PDLKGM L DMF	RELTDVRRYW	
rhesus m.	LQGS M D LLQ	GVDG I IKRIE	NMTLKKPKTF	HKNQRRVFRA	PDLKGM L DMF	RELTD A RRYW	
squirrel m.	LQGS V VELLQ	DVDGVIKRIE	<u>KVTLQ</u> KPKTF	<u>LNEKRR</u> VFRA	PDLK RML Q V L	<u>KELTE</u> VQRYW	
tamarin	LQGS V LELLQ	GVD DVIKRIE	<u>TVT LQ</u> KPKTF	<u>LNEKRR</u> VFRA	PDLK AML Q A F	<u>KELTE</u> VQRYW	
spider m.	LQGSVMELLQ	D V EGVIKRIK	NVTLQ KPKTF	<u>LNEKRR</u> VFRA	PDLKGM L Q V F	<u>KGLKE</u> VQCYW	

FIG. 1. Primate TRIM5 α sequences. The primary amino acid sequences of the TRIM5 α proteins studied herein are aligned. The primate species of origin are indicated to the left of the sequences (agm, African green monkey; pyg, subspecies pygerythrus; tan, subspecies tantalus; m., monkey). Amino acid residues that exhibit variation among the TRIM5 α proteins are colored blue in the hominoids, green in the Old World monkeys, and red in the New World monkeys. Gaps in the sequence are indicated by dots. The boundaries of the RING, B-box 2, coiled-coil, and B30.2 (SPRY) domains are indicated. The locations of variable regions (v1, v2, and v3) within the B30.2 (SPRY) domain are indicated (33a). The tandemly duplicated sequences within the v1 regions of African green monkey TRIM5 α proteins are underlined in green and black. The tandemly triplicated sequences in the TRIM5 α protein of spider monkeys are underlined in red. The v4 variable region is not marked.

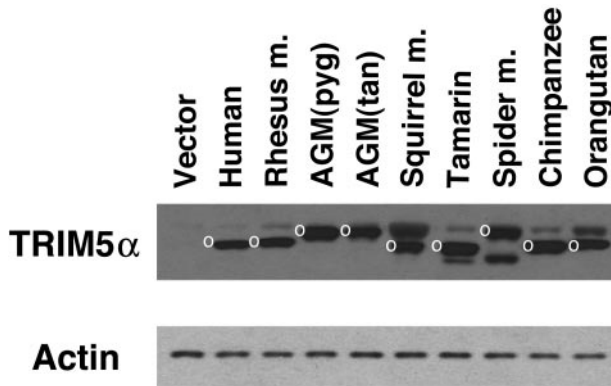


FIG. 2. Expression of the primate TRIM5 α proteins. Lysates from HeLa cells stably expressing the indicated primate TRIM5 α proteins, which contained HA epitope tags, were subjected to Western blotting with an antibody against HA. Control lysates from HeLa cells transduced with the empty pLPCX vector were analyzed in parallel (Vector). The major band corresponding to the TRIM5 α protein is indicated by a white circle. The lysates were also Western blotted with an antibody directed against β -actin.

ification were evident. The nature of these bands is under investigation.

Restriction of primate immunodeficiency virus infection by primate TRIM5 α proteins. The HeLa cells expressing the TRIM5 α proteins of different primate species and control HeLa cells transduced with the empty pLPCX vector were incubated with recombinant viruses containing the GFP gene. The recombinant viruses were derived from HIV-1, SIV_{mac}, SIV_{agm}, and MoMLV proviruses. The efficiency of infection was monitored by measuring the percentage of GFP-positive target cells.

As has been previously observed (37), human TRIM5 α moderately inhibited HIV-1-GFP infection but did not significantly affect SIV_{mac}-GFP infection (Fig. 3). Human TRIM5 α did not inhibit SIV_{agm}-GFP infection. The patterns of restriction observed for the chimpanzee and orangutan TRIM5 α proteins were similar to that of human TRIM5 α . Rhesus monkey TRIM5 α potently restricted infection by HIV-1-GFP and SIV_{agm}-GFP and partially blocked SIV_{mac}-GFP infection. Both African green monkey TRIM5 α proteins restricted HIV-1-GFP infection, but only TRIM5 α from the *C. aethiops tan-*

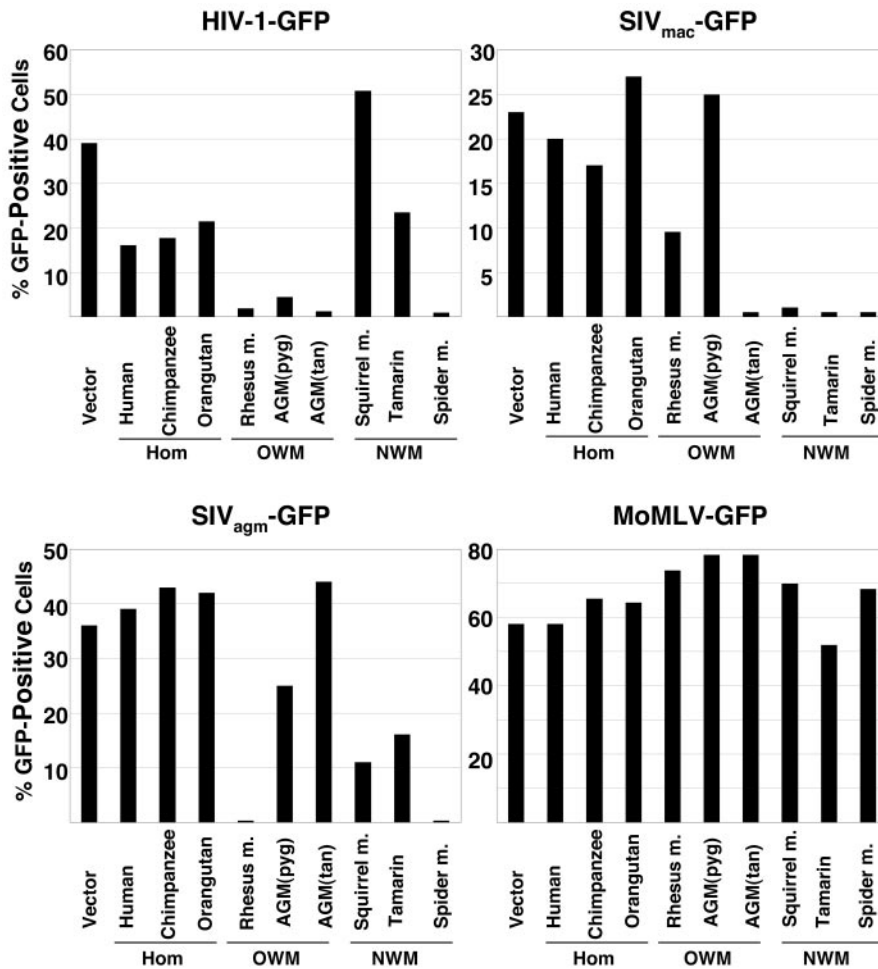


FIG. 3. Effect of expression of the primate TRIM5 α proteins on retrovirus infection. HeLa cells expressing TRIM5 α proteins from the various primate species, or control HeLa cells transduced with the empty pLPCX vector (Vector), were incubated with the indicated recombinant viruses expressing GFP. Infected, GFP-positive cells were counted by FACS. The results of a typical experiment are shown. Similar results were obtained in three independent experiments. Hom, hominoid; OWM, Old World monkey; NWM, New World monkey.

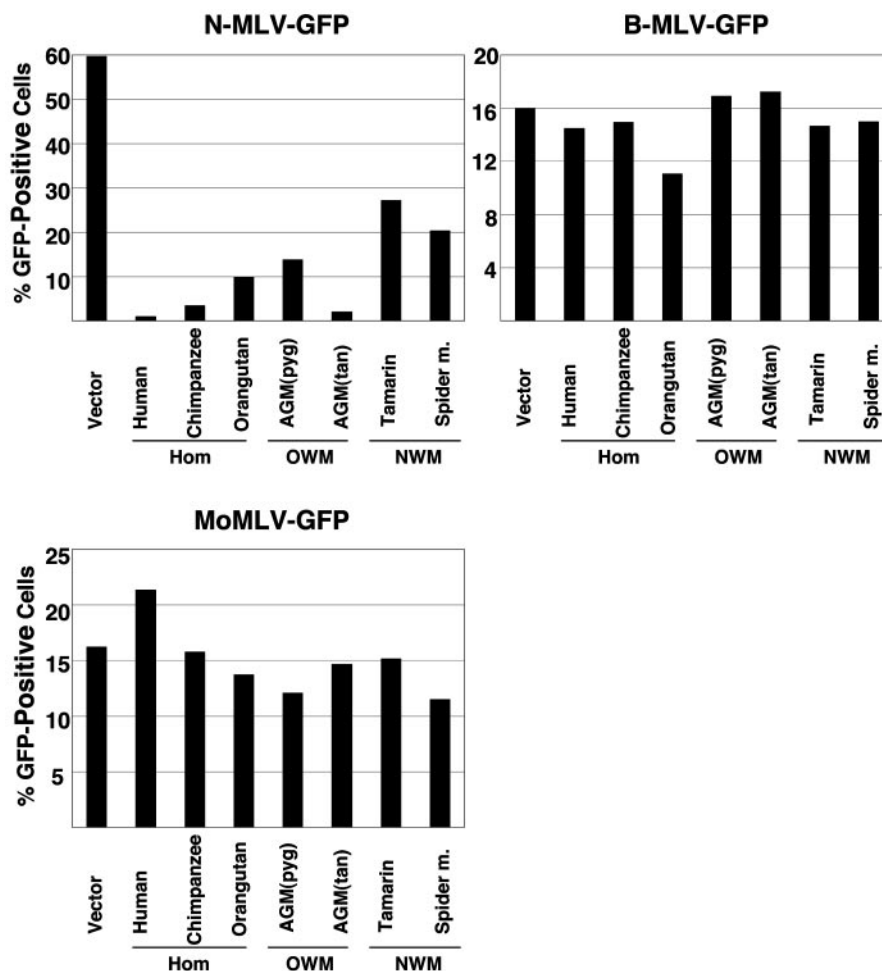


FIG. 4. Effect of expression of the primate TRIM5 α proteins on infection of different MLVs. NIH3T3 cells expressing TRIM5 α proteins from the various primate species, or control HeLa cells transduced with the empty pLPCX vector (Vector), were incubated with the indicated recombinant viruses expressing GFP. Infected, GFP-positive cells were counted by FACS. The results of a typical experiment are shown. Similar results were obtained in three independent experiments. Hom, hominoid; OWM, Old World monkey; NWM, New World monkey.

talus subspecies blocked SIV_{mac}-GFP infection. Neither African green monkey TRIM5 α protein significantly inhibited infection by SIV_{agm}-GFP viruses.

The TRIM5 α proteins from all three New World monkey species examined in this study potently restricted SIV_{mac}-GFP infection (Fig. 3). The spider monkey TRIM5 α protein also efficiently blocked HIV-1-GFP and SIV_{agm}-GFP infections. Modest inhibition of SIV_{agm}-GFP infection was observed for squirrel monkey and tamarin TRIM5 α proteins; the latter TRIM5 α protein also partially blocked HIV-1-GFP infection.

MoMLV-GFP infection was not significantly affected by expression of any of the primate TRIM5 α proteins examined (Fig. 3), indicating the specificity of the observed inhibitory effects on infection by primate immunodeficiency viruses.

Restriction of N-MLV infection by primate TRIM5 α proteins. The NIH3T3 cells expressing the different primate TRIM5 α proteins were incubated with recombinant murine leukemia viruses N-MLV-GFP, B-MLV-GFP, and MoMLV-GFP. The percentage of GFP-positive target cells was used as an indication of the efficiency of infection.

All of the primate TRIM5 α proteins inhibited N-MLV-GFP

infection, at least moderately (Fig. 4). The TRIM5 α proteins from humans, chimpanzee, orangutan, and African green monkeys significantly reduced the efficiency of N-MLV-GFP infection. The New World monkey TRIM5 α proteins less potently inhibited N-MLV-GFP infection. None of the TRIM5 α proteins tested significantly affected the efficiency of B-MLV-GFP or MoMLV-GFP infection.

DISCUSSION

Considerable variation among TRIM5 α proteins of different primate species exists (33a). This observation is consistent with expectations of variation in host molecules that coevolve with the infectious agents targeted. Despite the observed variation, all of the cloned TRIM5 α proteins retain the ability to suppress infection by at least one of the retroviruses tested. Key elements of the TRIM domains, such as the cysteines and histidines of the RING domain, are preserved in all of the primate TRIM5 α proteins. RING domain cysteines have been shown to contribute to the anti-HIV-1 activity of rhesus monkey TRIM5 α (37).

The patterns of postentry restrictions on the infection of several retroviruses in the cells of various primate species can be explained by TRIM5 α variation. HIV-1 infection is blocked soon after entry into Old World monkey cells (14, 15, 32); the TRIM5 α proteins from these monkeys potently restrict HIV-1 infection. SIV_{mac} infection encounters postentry restrictions in most New World monkey cells (15). The TRIM5 α proteins of three species from distinct branches of the New World monkey phylogenetic tree potently blocked SIV_{mac} infection. Although New World monkeys have not been exhaustively characterized with respect to postentry restriction of immunodeficiency virus infection, owl monkeys represent the only known exception to the block to SIV_{mac} in the New World lineage of monkeys (15). The owl monkey *TRIM5* has recently been shown to be disrupted by a retrotransposon-mediated insertion, precluding expression of the complete TRIM5 α protein in this species (24, 30). Combined with our data, this observation suggests that TRIM5 α is the major postentry restriction factor for SIV_{mac} in New World monkey cells. Given the current geographic isolation of New World monkeys from known SIVs, TRIM5 α activity against SIV_{mac} in these species may have been selected by past viral epidemics.

N-MLV infection is restricted at a postentry level in the cells of some but not all primates (4, 40). TRIM5 α accounts for this restriction in the cells of humans and African green monkeys (12, 17, 27, 47). Rhesus monkey cells do not restrict N-MLV infection after virus entry (4, 39), consistent with the less potent ability of the TRIM5 α protein from this species to block N-MLV infection. We observed that the TRIM5 α proteins of chimpanzees and orangutans efficiently inhibited N-MLV infection, suggesting that the cells of some apes will restrict infection by this virus. A gibbon cell line has been reported to be susceptible to N-MLV infection (4, 39), suggesting that quantitative or qualitative differences in TRIM5 α expression or antiviral function in some ape cells exist. The TRIM5 α proteins of New World monkeys exhibited only moderate inhibition of N-MLV infection. This is consistent with the reported ability of marmoset cells to support the early, postentry steps in N-MLV infection (4, 39). As expected, owl monkey cells, which do not express a complete TRIM5 α protein (24, 30), are also susceptible to infection by recombinant N-MLV vectors. Further work will be required to determine if the sporadic restriction of N-MLV infection observed in some mammalian species other than primates (4, 39) is mediated by TRIM5-related proteins.

Some of the observations made in this and related studies (12, 17, 27, 47) support a role for TRIM5 α in determining the species tropism of retroviruses in vivo. In no instance does a retrovirus naturally infect a host that encodes a TRIM5 α protein with potent restricting activity against the virus. The ubiquitous expression of the TRIM5 α protein in many cells of the host (28) probably contributes to the strength of this correlation. Moreover, it appears that naturally infecting immunodeficiency viruses may have evolved to reduce the impact of the TRIM5 α protein of the preferred host species to an acceptable minimum. Thus, HIV-1 demonstrates only moderate sensitivity to the TRIM5 α proteins of humans and chimpanzees. SIV_{agm} is relatively resistant to inhibition by the TRIM5 α proteins from two subspecies of African green monkey. Likewise, SIV_{mac} is only moderately inhibited by the rhesus monkey

TRIM5 α protein; although rhesus macaques are an Asian species and not naturally infected by SIVs, they are related to mangabeys (*Cercocebus* species), the natural host of SIV_{smm}, from which SIV_{mac} was derived (8, 9, 31). The TRIM5 α proteins of rhesus macaques and mangabeys are phylogenetically related (33a).

The TRIM5 α proteins of the different primate species were all efficiently expressed in the transduced HeLa cells. Therefore, most of the observed specificity for particular retroviruses likely arises from intrinsic differences in the TRIM5 α proteins themselves. The most dramatic variation among primate TRIM5 α proteins occurs in the B30.2 (SPRY) domain, which has been shown to be essential for the ability of TRIM5 α to restrict HIV-1 infection (37). Comparative analysis of multiple TRIM proteins has revealed the existence of four variable regions within the B30.2 domain (33a). Considerable length polymorphism in the v1 and v3 variable regions characterizes the TRIM5 α proteins of primates. The v1 region of Old World primates is relatively long (33a); the v1 region of TRIM5 α has been shown to be the major determinant of anti-HIV-1 potency of that protein (38). The v3 region of New World primates is relatively long (33a); this feature might contribute to the potent restriction of SIV_{mac} observed for these TRIM5 α variants. An attractive model is that the variable regions of the B30.2 domain represent surface-exposed loops that mediate contacts with the targeted viral capsid. Such a model is supported by the observation that owl monkeys do not express TRIM5 α but instead express TRIMCyp, a fusion protein in which the TRIM5 α B30.2 domain is replaced by cyclophilin A (24, 30). TRIMCyp acquires the ability to restrict HIV-1 infection by virtue of the binding of the HIV-1 capsid by the cyclophilin A moiety (24, 30). Thus, carboxy-terminal elements that contact retroviral capsids may be functionally complemented for antiviral activity by the TRIM5 amino-terminal elements.

Consistent with a contribution of the B30.2 variable regions to antiretroviral specificity is the observation that the TRIM5 α variants with the broadest range of targeted viruses exhibited unusually long variable regions. The African green monkey TRIM5 α v1 region is 46 residues long, 18 residues longer than the corresponding region of most Old World monkey TRIM5 α proteins (33a). African green monkey TRIM5 α variants can restrict HIV-1, SIV_{mac}, N-MLV, and equine infectious anemia virus (12, 17, 47). These restrictions, although broad in range, are not nonspecific, as the SIV_{agm} virus, as well as B-MLV and MoMLV, are not inhibited. Moreover, SIV_{mac} infection is potently blocked by TRIM5 α _{agm(tan)} but is not affected by the closely related TRIM5 α _{agm(pyg)}. Thus, subtle sequence differences between viruses and TRIM5 α proteins can exert significant effects on the efficiency of restriction.

The spider monkey TRIM5 α v3 region is 96 residues long, much larger than the length of 41 residues typical of New World monkey v3 regions (33a). Like the long v1 region of the African green monkey TRIM5 α protein, the spider monkey TRIM5 α v3 region apparently arose by the generation of tandem repeat sequences. The spider monkey TRIM5 α protein was able to inhibit HIV-1, SIV_{mac}, SIV_{agm} and, partially, N-MLV. Long B30.2 variable loops may provide flexible structures and multiple sequence motifs, allowing interaction with several different viral capsids.

Future studies should clarify the interactions of TRIM5 α

proteins and retrovirus capsids and might suggest avenues for intervention in retroviral infections.

ACKNOWLEDGMENTS

We thank Yvette McLaughlin and Sheri Farnum for manuscript preparation.

This work was supported by grants from the National Institutes of Health (HL54785 and AI063987) and by a Center for AIDS Research grant (PO30 AI28691). We also acknowledge the support of the Bristol-Myers Squibb Foundation, the International AIDS Vaccine Initiative, and the late William F. McCarty-Cooper.

REFERENCES

- Artes, E. J., and M. A. Wainberg. 1996. Human immunodeficiency type 1 reverse transcriptase and early events in reverse transcription. *Adv. Virus Res.* **46**:97–163.
- Benveniste, R. E., R. Heinemann, G. L. Wilson, R. Callahan, and G. J. Todaro. 1974. Detection of baboon type C viral sequences in various primate tissues by molecular hybridization. *J. Virol.* **14**:56–67.
- Besnier, C., Y. Takeuchi, and G. Towers. 2002. Restriction of lentivirus in monkeys. *Proc. Natl. Acad. Sci. USA* **99**:11920–11925.
- Besnier, C., L. Ylinen, B. Strang, A. Lister, Y. Takeuchi, S. P. Goff, and G. J. Towers. 2003. Characterization of murine leukemia virus restriction in mammals. *J. Virol.* **77**:13403–13406.
- Beyer, W. R., M. Westphal, W. Ostertag, and D. von Laer. 2002. Oncoretrovirus and lentivirus vectors pseudotyped with lymphocytic choriomeningitis virus glycoprotein: vector concentration, and broad host range. *J. Virol.* **76**:1488–1495.
- Bieniasz, P. D. 2003. Restriction factors: a defense against retroviral infection. *Trends Microbiol.* **11**:286–291.
- Cowan, S., T. Hatzioannou, T. Cunningham, M. A. Muesing, H. G. Gottlinger, and P. D. Bieniasz. 2002. Cellular inhibitors with Fv1-like activity restrict human and simian immunodeficiency virus tropism. *Proc. Natl. Acad. Sci. USA* **99**:11914–11919.
- Daniel, M. D., N. L. Letvin, N. W. King, M. Kannagi, P. K. Sehgal, R. D. Hunt, P. J. Kanki, M. Essex, and R. C. Desrosiers. 1985. Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. *Science* **228**:1201–1204.
- Desrosiers, R. C. 1990. The simian immunodeficiency viruses. *Annu. Rev. Immunol.* **8**:557–578.
- Hatzioannou, T., S. Cowan, S. P. Goff, P. D. Bieniasz, and G. J. Towers. 2003. Restriction of multiple divergent retroviruses by Lv1 and Ref1. *EMBO J.* **22**:385–394.
- Hatzioannou, T., S. Cowan, U. K. von Schwedler, W. I. Sundquist, and P. Bieniasz. 2004. Species-specific tropism determinants in the human immunodeficiency virus type 1 capsid. *J. Virol.* **78**:6005–6012.
- Hatzioannou, T., D. Perez-Caballero, A. Yang, S. Cowan, and P. D. Bieniasz. 2004. Retrovirus resistance factors Ref1 and Lv1 are species-specific variants of TRIM5. *Proc. Natl. Acad. Sci. USA* **101**:10774–10779.
- Henry, J., I. H. Mather, M. F. McDermott, and P. Pontarotti. 1998. B30.2-like domain proteins: update and new insights into a rapidly expanding family of proteins. *Mol. Biol. Evol.* **15**:1696–1705.
- Himathongkham, S., and P. A. Luciw. 1996. Restriction of HIV-1 (subtype B) replication at the entry step in rhesus macaque cells. *Virology* **219**:485–488.
- Hofmann, W., D. Schubert, J. LaBonte, L. Munson, S. Gibson, J. Scammell, P. Ferrigno, and J. Sodroski. 1999. Species-specific, postentry barriers to primate immunodeficiency virus infection. *J. Virol.* **73**:10020–10028.
- Johnson, W. E., and J. M. Coffin. 1999. Constructing primate phylogenies from ancient retrovirus sequences. *Proc. Natl. Acad. Sci. USA* **96**:10254–10260.
- Keckesova, Z., L. M. J. Ylinen, and G. J. Towers. 2004. The human and African green monkey TRIM genes encode Ref1 and Lv1 retroviral restriction factor activities. *Proc. Natl. Acad. Sci. USA* **101**:10780–10785.
- Kim, H. S., O. Takenaka, and T. J. Crow. 1999. Isolation and phylogeny of endogenous retrovirus sequences belonging to the HERV-W family in primates. *J. Gen. Virol.* **80**:2613–2619.
- Kootstra, N. A., C. Munk, N. Tonnu, N. R. Landau, and I. M. Verma. 2003. Abrogation of postentry restriction of HIV-1-based lentiviral vector transduction in simian cells. *Proc. Natl. Acad. Sci. USA* **200**:1298–1303.
- Mager, D. L., and J. D. Freeman. 1995. HERV-H endogenous retroviruses: presence in the New World branch but amplification in the Old World primate lineage. *Virology* **213**:395–404.
- Mang, R., J. Maas, A. C. van der Kuyl, and J. Goudsmit. 2000. *Papio cynocephalus* endogenous retrovirus among Old World monkeys: evidence for coevolution and ancient cross-species transmission. *J. Virol.* **74**:1578–1586.
- Mariana-Costantini, R., T. M. Horn, and R. Callahan. 1989. Ancestry of a human endogenous retrovirus family. *J. Virol.* **63**:4982–4985.
- Munk, C., S. M. Brandt, G. Luccero, and N. R. Landau. 2002. A dominant block to HIV-1 replication at reverse transcription in simian cells. *Proc. Natl. Acad. Sci. USA* **99**:13843–13848.
- Nisole, S., C. Lynch, J. P. Stoye, and M. W. Yap. 2004. A Trim5-cyclophilin A fusion protein found in owl monkey kidney cells can restrict HIV-1. *Proc. Natl. Acad. Sci. USA* **101**:13324–13328.
- Owens, C. M., B. Song, M. J. Perron, P. C. Yang, M. Stremlau, and J. Sodroski. 2004. Binding and susceptibility to post-entry restriction factors in monkey cells are specified by distinct regions of the human immunodeficiency virus type 1 capsid. *J. Virol.* **78**:5423–5437.
- Owens, C. M., P. C. Yang, H. Gottlinger, and J. Sodroski. 2003. Human and simian immunodeficiency virus capsid proteins are major viral determinants of early, postentry replication blocks in simian cells. *J. Virol.* **77**:726–731.
- Perron, M., M. Stremlau, B. Song, W. Ulm, R. Mulligan, and J. Sodroski. 2004. TRIM5 α mediates the postentry block to N-tropic murine leukemia viruses in human cells. *Proc. Natl. Acad. Sci. USA* **101**:11827–11832.
- Reymond, A., G. Meroni, A. Fantozzi, G. Merla, S. Cairo, L. Luzi, D. Riganelli, E. Zanaria, S. Messali, S. Cainarca, A. Guffanti, P. G. Pelicci, and A. Ballabio. 2001. The tripartite motif family identifies cell compartments. *EMBO J.* **20**:2140–2151.
- Rho, H. M., B. Poiesz, F. W. Ruscetti, and R. C. Gallo. 1981. Characterization of the reverse transcriptase from a new retrovirus (HTLV) produced by a human cutaneous T-cell lymphoma cell line. *Virology* **112**:355–360.
- Sayah, D. M., E. Sokolskaja, L. Berthoux, and J. Luban. 2004. Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. *Nature* **430**:569–573.
- Sharp, P. M., E. Bailes, R. R. Chaudhuri, C. M. Rodenburg, M. O. Santiago, and B. H. Hahn. 2001. The origins of acquired immune deficiency syndrome viruses: where and when? *Philos. Trans. R. Soc. London B* **356**:867–876.
- Shibata, R., H. Sakai, M. Kawamura, K. Tokunaga, and A. Adachi. 1995. Early replication block of human immunodeficiency virus type 1 in monkey cells. *J. Gen. Virol.* **76**:2723–2730.
- Soares, M., D. L. Robertson, H. Hui, J. S. Allan, G. M. Shaw, and B. H. Hahn. 1997. A full-length and replication-competent proviral clone of SIVAGM from tanzania monkeys. *Virology* **228**:394–399.
- Song, B., B. Gold, C. O'Uigin, H. Javanbakht, X. Li, M. Stremlau, C. Winkler, M. Dean, and J. Sodroski. 2005. The B30.2(SPRY) domain of the retroviral restriction factor TRIM5 α exhibits lineage-specific length and sequence variation in primates. *J. Virol.*, in press.
- Steele, P. E., M. A. Martin, A. B. Rabson, T. Bryan, and S. J. O'Brien. 1986. Amplification and chromosomal dispersion of human endogenous retroviral sequences. *J. Virol.* **59**:545–550.
- Steinhuber, S., M. Brack, G. Hunsmann, H. Schwelberger, M. P. Dierich, and W. Vogetseder. 1995. Distribution of human endogenous retrovirus HERV-K genomes in humans and different primates. *Hum. Genet.* **96**:188–192.
- Stoye, J. P. 2002. An intracellular block to primate lentivirus replication. *Proc. Natl. Acad. Sci. USA* **99**:11549–11551.
- Stremlau, M., C. M. Owens, M. J. Perron, M. Kiessling, P. Autissier, and J. Sodroski. 2004. The cytoplasmic body component TRIM5 α restricts human immunodeficiency virus (HIV-1) infection in Old World monkeys. *Nature* **427**:848–853.
- Stremlau, M., M. Perron, S. Welikala, and J. Sodroski. 2005. Species-specific variation in the B30.2(SPRY) domain of TRIM5 α determines the potency of human immunodeficiency virus restriction. *J. Virol.* **79**:3139–3145.
- Towers, G., M. Bock, S. Martin, Y. Takeuchi, J. P. Stoye, and O. Danos. 2000. A conserved mechanism of retrovirus restriction in mammals. *Proc. Natl. Acad. Sci. USA* **97**:12295–12299.
- Towers, G., M. Collins, and Y. Takeuchi. 2002. Abrogation of Ref1 retrovirus restriction in human cells. *J. Virol.* **76**:2548–2550.
- Towers, G. J., T. Hatzioannou, S. Cowan, S. P. Goff, J. Luban, and P. D. Bieniasz. 2003. Cyclophilin A modulates the sensitivity of HIV-1 to host restriction factors. *Nat. Med.* **9**:1138–1143.
- Tristem, M. 2000. Identification and characterization of novel human endogenous retrovirus families by phylogenetic screening of the human genome mapping project database. *J. Virol.* **74**:3715–3730.
- van der Kuyl, A. C., J. T. Dekker, and J. Goudsmit. 1995. Distribution of baboon endogenous virus among species of African monkeys suggests multiple ancient cross-species transmissions in shared habitats. *J. Virol.* **69**:7877–7887.
- van der Kuyl, A. C., R. Mang, J. T. Dekker, and J. Goudsmit. 1997. Complete nucleotide sequence of simian endogenous type D retrovirus with intact genome organization: evidence for ancestry to simian retrovirus and baboon endogenous virus. *J. Virol.* **71**:3666–3676.
- Whitcomb, J. M., and S. H. Hughes. 1992. Retroviral reverse transcription and integration: progress and problems. *Annu. Rev. Cell Biol.* **8**:275–306.
- Wilkinson, D. A., D. L. Mager, and J.-A. C. Leong. 1994. Endogenous human retroviruses, p. 465–535. *In* J. A. Levy (ed.), *The Retroviridae*, vol. III. Plenum Press, New York, N.Y.
- Yap, M. W., S. Nisole, C. Lynch, and J. P. Stoye. 2004. Trim5 protein restricts both HIV-1 and murine leukemia virus. *Proc. Natl. Acad. Sci. USA* **101**:10786–10791.
- Yee, J. K., T. Friedmann, and J. C. Burns. 1994. Generation of high-titer pseudotyped retroviral vectors with very broad host range. *Methods Cell Biol.* **43**:99–112.