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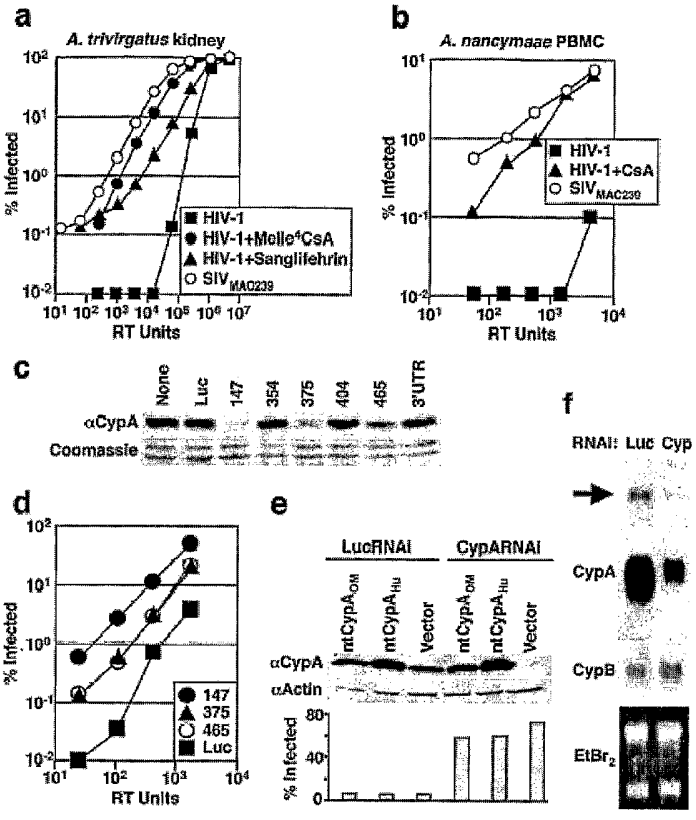
(54) Title: POLYNUCLEOTIDE ENCODING A TRIM-CYP POLYPEPTIDE, COMPOSITIONS THEREOF, AND METHODS OF USING SAME



(57) Abstract: The invention provides an isolated nucleic acid encoding a TRIM - cyclophilin A fusion sequence encoding a TRIMcyp fusion protein which is active as an anti-viral agent, and in particular an anti-HIV-1 agent. The invention provides for a nucleic acid encoding a polypeptide having both TRIM activity and cyclophilin activity. The invention provides for an isolated polynucleotide encoding a TRIM-cyclophilin fusion protein, or variants thereof retaining the TRIM and cyclophilin activities. The invention provides for compositions thereof, antibodies that specifically bind thereto, and vectors and host cells comprising the nucleic acid or polypeptide. In addition, the invention provides for methods for treating or preventing viral infection, or reducing viral load in a subject comprising administering the nucleic acid, polypeptide, vector, or composition to the subject in an amount effective to treat or prevent the viral infection. In some embodiments, the viral infection is HIV-1 infection, hepatitis C infection, pox virus infection, vaccinia virus infection, or HTLV infection.

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POLYNUCLEOTIDE ENCODING A TRIM-CYP POLYPEPTIDE, COMPOSITIONS THEREOF, AND METHODS OF USING SAME

[0001] This application claims priority to U.S. Serial No. 60/585,925, filed on July 6, 2004, which is hereby incorporated by reference in its entirety.

[0002] The invention disclosed herein was made with Government support under NIH Grant No. RO1 A1036199-011 from the Department of Health and Human Services. Accordingly, the U.S. Government has certain rights in this invention.

[0004] Throughout this application, patent applications, published patent applications, issued and granted patents, texts, and literature references are cited. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

[0005] Background of the Invention

[0006] In Old World primates, TRIM5-α confers a potent block to human immunodeficiency virus type 1 (HIV-1) infection that acts after virus entry into cells (1-5). (References are listed in numerical order at the end of the specification, and are all incorporated by reference in their entireties for all purposes.) Cyclophilin A (CypA) binding to viral capsid protects HIV-1 from a similar activity in human cells (4, 6-8). Among New World primates, only owl monkeys exhibit post-entry restriction of HIV-1 (1). Paradoxically, the barrier to HIV-1 in owl monkey cells is released by capsid mutants or drugs that disrupt capsid interaction with CypA (4). HIV-1 infection is a serious problem throughout the world and there is a great need for a composition that will prevent infection of a subject by HIV-1 and for a composition that treats or ameliorates the effects of HIV-1 infection in humans.

Summary Of The Invention

[0007] The present invention provides an isolated nucleic acid which comprises consecutive nucleotides having a sequence selected from the group consisting of: SEQ ID NO:1 (TRIM-

Cyp sequence), SEQ ID NO:2 (Aotus TRIM5 Locus (partial genomic sequence)), SEQ ID NO:3 (OMK CypA cDNA), and a variant of any one of the SEQ ID NOS having at least about 50% identity to the SEQ ID NO, and encoding a polypeptide having both TRIM activity and cyclophilin activity. The invention provides for an isolated nucleic acid which comprises consecutive nucleotides having a sequence complementary to the nucleic acids shown in SEQ ID NOS: 1-3. In one embodiment, the invention provides for a nucleic acid which is a variant that has at least about 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identity to any one of SEQ ID NO: 1, 2 or 3, as determined by analysis with a sequence comparison algorithm. The invention also provides for an isolated nucleic acid that hybridizes to such a nucleic acid under high stringency, moderate stringency, or low stringency.

[0008] The invention provides for an isolated nucleic acid encoding a polypeptide comprising consecutive amino acids having a sequence comprising SEQ ID NO: 4. The invention also provides for an isolated polypeptide encoded by any of the aforementioned nucleic acids. The invention provides for a purified polypeptide substantially identical to the described polypeptides as determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 using default parameters. In one embodiment, the invention provides for a polypeptide having the amino acid sequence of SEQ ID NO:4.

[0009] The invention provides for an isolated polynucleotide encoding a TRIM-cyclophilin fusion protein. In one embodiment the polynucleotide encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:4. In another embodiment, the polynucleotide encodes a polypeptide consisting essentially of the amino acid sequence of SEQ ID NO:4. In another embodiment, the polynucleotide encodes a polypeptide comprising a sequence that is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical to SEQ ID NO:4 and the polypeptide has the function of TRIM and specifically binds a capsid protein of HIV.

[0010] The invention provides for a purified antibody that specifically binds to a polypeptide of the invention. In one embodiment, the antibody is a polyclonal antibody, a monoclonal antibody, or a chimeric antibody. In another embodiment, the antibody specifically binds to the protein encoded by any of the aforementioned polynucleotides.

[0011] The invention provides for a method of producing a polypeptide of the invention which comprises: (a) introducing a nucleic acid encoding the polypeptide into a host cell

under conditions that permit expression of the polypeptide by the host cell, and (b) recovering the polypeptide.

[0012] The invention also provides for a replicable nucleic acid vector, which comprises a nucleic acid of the invention. In one embodiment, the vector comprises a viral vector or a retroviral vector. In another embodiment, the vector is an adenovirus vector, a retroviral vector, or an adeno-associated viral (AAV) vector. In another embodiment, the invention provides for a host organism comprising the replicable vector of the invention. In one embodiment, the host is a prokaryote, a eukaryote, or a fungus.

[0013] The invention also provides for a method for preparing a pharmaceutical composition which comprises admixing a polypeptide or a fragment thereof of the invention, thereby preparing the pharmaceutical composition. In one embodiment, the pharmaceutical composition comprises a nucleic acid of the invention, a polypeptide of the invention, or a vector of the invention, and a carrier.

[0014] In one embodiment, the invention provides for a method for treating a subject suffering from a disease or condition, which comprises administering to the subject a nucleic acid of the invention, a polypeptide of the invention, a vector of the invention, or a pharmaceutical composition of the invention.

[0015] The invention provides for a method for treating a subject suffering from a disease or a condition, which comprises administering to the subject a polypeptide or a fragment thereof of the invention, so as to treat the subject.

[0016] The invention provides for a method for preventing retroviral infection of a subject, or for treating a subject with retroviral infection, which comprises administering to the subject a pharmaceutical composition of the invention.

[0017] The invention provides for a method for treating or preventing a viral infection of a cell which comprises introducing a TRIM-Cyp fusion polypeptide into the cell. In one embodiment, the introducing comprises tranfection of a polynucleotide encoding a TRIM-Cyp fusion polypeptide, transduction, viral-mediated introduction, or liposome-mediated introduction. In another embodiment, the viral infection is an HIV infection, an HTLV infection, a pox virus infection, a retrovirus infection, a malaria infection, a hepatitis C virus,

a hepatitis B virus, or a vaccinia virus infection. In another embodiment, the pox virus is small pox.

[0018] The invention provides a method for treating HIV infection or preventing HIV infection of a subject which comprises introducing into cells of the subject a TRIM-Cyp polypeptide. In one embodiment, the TRIM-Cyp polypeptide binds to a capsid protein of the HIV and subsequently degrades the HIV, thereby treating or preventing HIV infection in the subject.

[0019] The invention also provides a peptidomimetic comprising an amino acid sequence substantially identical to the amino acid sequence of a polypeptide of the invention and wherein the peptidomimetic comprises TRIM function and cyclophilin function.

[0020] The invention provides for a stem cell comprising a nucleic acid of the invention, wherein the nucleic acid is part of the genome of the stem cell.

[0021] The invention provides for a method of imparting resistance to HIV to a cell or a subject which comprises administering to the cell or to the cells of the subject a nucleic acid of the invention, a vector of the invention, or a polypeptide of the invention in an amount effective to impart resistance to HIV infection of cells in the subject.

[0022] The invention provides for a therapeutic composition comprising a nucleic acid of the invention, a polypeptide of the invention, or a peptidomimetic of the invention, and a therapeutically acceptable carrier. In one embodiment, the carrier comprises a vector, a liposome, or a viral vector.

[0023] The invention provides for a method of *ex vivo* gene therapy which comprises: (a) removing bone marrow cells from a subject; (b) transfecting the removed bone marrow cells with a nucleic acid or vector of the invention *in vitro*; and (c) transplanting the transfected bone marrow back into the subject.

[0024] The invention provides for a method for reducing viral burden or load in a subject infected by a virus which comprises administering to the subject a nucleic acid of the invention, a polypeptide of the invention, or a vector of the invention.

[0025] The invention provides for a topical composition for prevention or treatment of a viral infection which comprises a nucleic acid of the invention or a polypeptide of the invention,

and a topical carrier. In one embodiment, the composition is for use on the skin, in the vagina, in the nose, in the mouth, or on any skin or muscosal surface.

[0026] The invention also provides for a method for reducing transposition events in a genome of a cell which comprises introducing into the cell a polynucleotide encoding TRIM-cyclophilin A fusion protein, or the protein encoded by the polynucleotide, so as to inhibit L1 elements and reduce transposition events in a cell.

Brief Description Of The Figures

[0027] The present invention is now illustrated in connection with the accompanying drawings. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of the specific embodiments presented herein. The invention is not to be construed as limited to the embodiments disclosed, but it is to be understood that these are non-limiting examples of the invention disclosed.

[0028] Figure 1. A CypA homologue is required for owl monkey restriction of HIV-1. **a, b,** *Aotus trivirgatus* OMK cells (**a**) or *Aotus nancymaae* peripheral blood mononuclear cells (**b**) were infected with GFP-transducing virions in the presence of CypA-binding drugs. GFP-positive cells were counted by flow cytometry. (RT units are reverse transcriptase units) **c, d,** OMK cells were transduced with retroviruses delivering shRNAs targeting CypA mRNA at the indicated nucleotide positions. Lysates were immunoblotted (**c**) and cells were challenged with HIV-1/GFP (**d**). After antibody detection, the membrane was Coomassie-stained and a representative section is shown as a loading control. Luc is the control shRNA targeting luciferase. **e**, OMK cells transduced with shRNA-147 were selected after transfection with non-targetable-CypA expression vectors (human or owl monkey) and immunoblotted (top), or infected with HIV-1/GFP (bottom). **f**, Northern blot of total cytoplasmic RNA from shRNA-147- or shRNA-Luc-treated OMK cells, probed with *CypA* and *CypB* cDNA. The arrow indicates a ~2-kb, RNAi-responsive mRNA that hybridizes to *CypA*. $EtBr_2$, ethidium bromide.

[0029] Figure 2. Owl monkey cells express a TRIM5–CypA fusion protein that blocks HIV-1 infection. **a**, Predicted amino-acid sequence of 54-kD TRIMCyp protein. (SEQ ID NO: 4) TRIM5 segment highlighted in grey with domains indicated above; CypA segment highlighted in black. **b**, Immunoblot of 293T cells transfected with TRIMCyp expression vector, probed for TRIM5 and CypA. **c, d**, RT–PCR for *TRIMCyp*, *CypA* or *TRIM5-α* in

OMK, human HeLa and Jurkat, and macaque FRhK4 cells (**c**), and PBMC from two *Aotus nancymaae* monkeys (**d**). **e**, Amplification plot of real time RT–PCR. The green curves show undiluted and threefold serial dilutions of $OMK_{MH\text{-}Luc}$ cDNA; the black curve is undiluted $OMK_{MH\text{-}CypA\text{-}147}$ cDNA; the yellow curves are no RT controls. (RT is 'no reverse transcriptase', Rn is 'normalized reporter signal' and 'R' should be 'R^2' and is the correlation coefficient for the diluted samples.) *GAPDH* amplification curves (not shown) were identical between samples. **f**, $OMK_{MH\text{-}CypA\text{-}147}$ cells transduced with the indicated RNAi-resistant cDNAs and infected with HIV-1.

[0030] Figure 3. HIV-1 is restricted in human or rat cells transduced with *TRIMCyp*. TE671 cells transduced with the indicated cDNAs were infected with HIV-1 (**a**) or SIV_{MAC239} (**b**). **c**, *TRIMCyp*-transduced TE671 cells were infected with HIV-1 ±CsA or with HIV-1 bearing the capsid G89V mutation. WT, wild type. HeLa cells (**d**) or Rat2 cells (**e**) transduced with the indicated cDNAs were infected with HIV-1. Results shown are typical of those obtained in three independent experiments.

[0031] Figure 4. *TRIMCyp* arose from retrotransposition of *CypA* cDNA into *TRIM5*. **a**, Part of the owl monkey *TRIM5* locus showing a complete, processed CypA cDNA inserted after exon 7. **b**, **c**, *TRIM5* genomic sequence flanking the 5′ end (**b**) or the 3′ end (**c**) of the *CypA* insertion aligned with homologous human sequence. *TRIMCyp* exons and the region homologous to human exon 8 are in capital letters, introns in lower case, and the *CypA* cDNA insertion highlighted in grey. The 'natural' *CypA* start codon, the *CypA/TRIMCyp* poly-adenylation signal and poly-A tail are underlined. The insertion is flanked by a 16-bp target site duplication (TSD), highlighted in black. *At, Aotus trivirgatus. Hs, Homo sapiens.*

[0032] Figure 5. CypA does not restore restriction in CypA deleted cells. Owl monkey cells were treated with shRNA that knocks down cyclophilin A expression, rescuing HIV-1 from restriction. Cyclophilin A protein was then added back to these cells but failed to restore the HIV-1 restriction. This result showed that the RNAi was targeting a protein other than cyclophilin A, which was necessary for HIV-1 restriction, and led to the cloning of TRIMCyp.

[0033] Figure 6. Inhibition of HIV-1 in human TE671 cells expressing owl monkey TRIMCyp or Rhesus macaque TRIM5α. LPCX-based retroviral vectors were used to transduce the indicated genes into human TE671 cells. Vect, Cyp, TS, TC and T5rh

designate, respectively, Vector control, owl monkey CypA, TRIMStop, TRIMCyp, and TRIM5α_{rh}. These cells were then infected for 16 h with HIV-$1_{NL\text{-}GFP}$ (pseudotyped vesicular stomatitis virus G protein) in the presence (+) or absence (-) of CsA (5 μM). One twentieth of the cells were maintained in culture for another day and used to determine the percentage of GFP-positive cells by FACS (bottom of the figure). Total DNA was extracted from the remainder of the cells 16 h after infection, and 5 μg of each DNA sample was analyzed by Southern blotting. The positions of the linear, 1-LTR, and 2-LTR HIV-1 cDNA species are indicated on the left. "Total" DNA refers to a band specific to all HIV-1 cDNA forms, including the integrated DNA.

[0034] Figure 7. TRIMCyp and TRIM5α_{rh} abrogate the antiviral activity of endogenous TRIM5α_{hu}. (**a**) TE671 cells expressing the indicated LPCX derived constructs were infected with GFP-expressing B-MLV and N-MLV vectors at multiple doses. Cells were infected in the presence or absence of As_2O_3 (3 μM). Two days later, the percentage of infected cells was determined by FACS. (**b**) OMK cells were transduced with MIG (Vect) or with MIG-TRIM5α_{hu} (T5hu) and challenged with DsRed-expressing N-MLV or B-MLV vectors. Two days later, the percentage of infected cells was determined by FACS. (**c**) OMK/MIG and OMK/MIG-T5hu cells were challenged with HIV-1-derived, DsRed-expressing CSRW vectors in the presence or absence of Cyclosporine A (2.5 μM).

[0035] Figure 8. Effect of TRIMStop on TRIM5α_{hu} antiviral activity. (**a**) TE671-Vector (Vector) cells and TE671-TRIMStop cells were infected with HIV-$1_{NL\text{-}GFP}$ (left panel) or with N- and B-MLV (right panel). Two days later, the percentage of infected (GFP expressing) cells was determined by FACS. (**b**) Total RNA was prepared from TE671-Vector (Vect), TE671-TRIMCyp (TC), and TE671-TRIMStop (TS), and RT-PCR was used to detect endogenous TRIM5α or TRIMCyp/TRIMStop. RT was performed in the presence (+) or absence (-) of reverse transcriptase. (**c**) 293T cells were transfected with LPCX (Vect), LPCX-TRIMCyp (TC), or LPCX-TRIMStop (TS), and Western blotting was performed using an antibody specific to TRIM5.

[0036] Figure 9. TRIMCyp and TRIMStop both bind TRIM5α_{hu}. (**a**) Yeast two-hybrid system. TRIM5α_{hu}, TRIM5α_{rh}, TRIMCyp, TRIMStop, huTRIMStop, and HIV-1 Gag were fused to *lexA*. TRIMCyp and TRIMStop were expressed in fusion with the B42 activation domain. Pairs of fused LexA and B42 expression plasmids were transformed into

Saccharomyces cerevisiae strain EGY48. For each transformant, β-galactosidase activity for three colonies is reported in Miller units with the standard deviation. (**b**) Binding in mammalian cells. TRIM5α_{hu} was fused to GST. 293T cells were transfected with GST or GST-TRIM5α_{hu} (GST-T5α) and cotransfected with LPCX (C), LPCX-TRIMCyp (TC), or LPCXTRIMStop (TS). Thirty-six hours later, the cells were lysed in 50 mM Tris-Cl (pH 8.0), 150 mM NaCl, 1% NP40, 0.1% SDS, and GST was pulled down using glutathione-coated Sepharose beads (Pharmacia). One percent of the pre-pull-down lysate and 25% of the bound proteins were analyzed by Western blotting, using polyclonal antibodies directed against TRIM5, cyclophilin A, or GST.

[0037] Figure 10. Colocalization of TRIMCyp and TRIMStop with TRIM5α_{hu}. TE671 cells were cotransfected with GST- TRIM5α_{hu} and with 3X FLAG N-terminal-tagged versions of either TRIMCyp or TRIMStop. Thirty-six hours later, cells were fixed with 4% formaldehyde, permeabilized with 0.1% Triton X-100, and probed with antibodies against GST (rabbit polyclonal; Chemicon International) and FLAG (mouse monoclonal; Sigma). Fluorescent staining was done using Alexa488-conjugated goat anti-mouse and Alexa594-conjugated goat anti-rabbit antibodies and Hoechst33342 (all from Molecular Probes) to reveal DNA. Pictures were generated using a Nikon TE300 microscope with the Openlab 3.0 software.

[0038] Figure 11. Human TRIM5α binds CA from restricted MLV virions. (**a**) HeLa cells were infected with VSV G-pseudotyped, N- and B-tropic MLV-GFP vectors after normalization for RT activity and infectivity on non-restrictive *Mus dunni* tail fibroblasts. The percentage of infected (GFP-positive) cells was determined by flow cytometry. (**b**) 293T cells were transfected with plasmids encoding glutathione S-transferase (GST) fusions with full-length TRIM5α or with TRIM5 lacking the SPRY domain. Cells were lysed (50 mM Tris pH 8.0, 150 mM NaCl, 1% NP-40, 0.1% SDS) and mixed for 2 hrs at 4°C with virions (N-MLV or B-MLV) that had been concentrated by acceleration through 25% sucrose. GST fusions and associated proteins were enriched on glutathione sepharose beads and immunoblotted with goat anti-MLV CA antibody (CA pull-out), or anti-GST antibody (bottom panel). Unbound CA remaining in the binding reaction was probed with anti-MLV CA antibody (CA input). TRIM5 protein domains fused to GST are indicated schematically on the bottom left: RF, ring finger; BB, B box; CC, coiled-coil.

[0039] Figure 12. CypA domain determines TrimCyp specificity. Human TRIM5 is fused to cyclophilin A to make the human equivalent to the owl monkey TRIM Cyp. The human version restricts HIV-1.

[0040] Figure 13. Human T cells are protected from a spreading, cytopathic HIV-1 infection by TRIMCyp. Retroviral vectors were used to transduce human Jurkat T cells with the cDNA encoding owl monkey TRIMCyp. Control cells were transduced with vector lacking the cDNA. Cells were challenged with full infectious HIV-1 clone NL4-3. Supernatant was collected and assessed for viral reverse transcriptase activity (a quantitative measure of the amount of virus produced in the culture).

Detailed Description

[0041] The following sequences are provided by this invention:

[0042] SEQ ID NO:1 (AY646198) - TRIMCyp cDNA

TGCAGGCCCCTGGATTGAGAATATAAACAACAATTCTTATTATCCCTTTTACTGG
TTTGCACGGGGAGAGAGAAGCCAAAGACCTGACTGGGATCTGTGAGCAAGAGGA
GCCTCAGCAGCCAGGACAGGCAAGAGTAGTGGAGCAGCTACTATGGCTTCCAGA
ATCCTGGTCAATATAAAGGAGGAGGTGACCTGCCCCATCTGCCTGGAACTCCTGA
CAGAACCCCTGAGCCTGGACTGTGGCCATAGCTTCTGCCAAGCATGCATCACTGC
AAATCACAAAAAGTCTATGCCACACCAAGGAGAGAGAAGCTGCCCTTTGTGCCG
GATCAGTTACTCGTCTGAGAACCTGCGGCCTAATCGGCATTTGGTCAACATAGTG
GAGAGGCTCAGGGAGGTCATGCTGAGCCCAGAGGAGGGGCAGAAGGTTGATCA
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ATTTGCTGGCTTTGTGAGCGGTCTCAAGAACACCGTGGGCACCAGACATTCCTTG
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TGAGGCAGAAGCAGAAGGATGCTGAAAAGTTGGAAGCTGACGTCAGAGAAGAG
CAAGCTTCCTGGAAGATTCAAATACAAAATGACAAAACCAACATCATGGCAGAG
TTTAAAAAACGGAGAGACATCCTGGACTGTGAGGAGAGCAAAGAGTTGCAAAAC
CTGGAGAAGGAGGAGAAAAACATTCTGAAAAGACTTGTACAGTCTGAAAATGAC
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CAATCCTACTGTGTTCTTCGACATTGCCGTCGATGGCGAGCCCTTGGGCCGCGTC
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GGTCCCGGTATCTTGTCCATGGCAAATGCTGGACCCAACACAAACGGTTCCCAGT
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GTATGGCAAGACCAGCAAAAAGATCACCATTGCTGACTGTGGACAACTTTAATA
AGTTTGACTTGTGTTTTGTCTTCACCACCAGACCATTCCTTCTGTAGCTCAGGAGA
GCACCCCTCCACCCCATTTGCTCGCAGTATCCTACAATCTGTGCTCTCGCTGCAGT
TCCCTTTGGGTTCCATGTTTTCCTTGTTCCCTTCCATGCCTAGCTGGATTGCAGAG
TTGAGTTTAAGTTTATGATTATGAAATAAAGACTAAATAACAAAAAAAAAAAAA
AAAAAAAAAAA

[0043] SEQ ID NO:2 (AY646199) - Aotus TRIM5 Locus (partial genomic sequence)

[0044] TTTCTTAATGAAAAAGGAGAATATTTCAAACTCCTGATCTGAAAGGAACA
CTACAAGTGTTTAAAGGTGAGGAGAGCTGGATCAACTGCGGGGTTGTGGAATGC
AAGTCCCGACTGTGTCAGGGTGCTAAATGGAGAAAAGAGTGTGGTTTCCAAATA
TGGATAGAGGGGATGGGGAAGATGGATATTATCTGCTGCTGATGGGATTATATTT
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ACCATAGGGCATGCCTACTCTTTCCCTAATCTGGAGAGAAAATGAATTTCAGTGC
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TTGGATGGCAAGCATGTGGTCTTTGGCAAGGTGAAAGAAGGCATGAATGTTGTG

GAGGCCATGGAGCGCTTTGGGTGCAGGTATGGCAAGACCAGCAAAAAGATCACC
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ATGTTTTAAGATTTATATTTCTTCTTCCAGCACACACATAACCTACCCTTCCTTAT
AACTTCTGAACAAGGTTCCTTCCAGTTTTCTTTCAAGGCTTTATTAAGATTTCTCT
CATATAATGTTATCCCTTACCTGACCTGTTAATTTTTTTACAGCTCATGTGACACT
GGTTCCAAGTCACCCTTCATGTACTGTCATTTCTGAAGATGAGAGACAAGTGAGA
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TGGGCTCCCCAAGTATCACATCAGGGAAACATTACTGGGAGGTAGACGTGTCCA
ATAAAAGTGAGTGGATCCTGGGGGTATGTGTTAGCTTGAAGCGCACTGCAAGTT
GTAGTGTTCCAAGAATTGAAAATGATCAACCTAAAAATGGCTACTGGGTTATAG
GGTTACGGAATGCAGATAACTATAGTGCTTTCCAGGATGCAGTTGAATATAGTGA
TTTCCAGGATGGTTCCCGCTCTACTCCTTCTGCTCCTTTGATCGTGCCCCTCTTTAT
GACTATTTGTCCTAATCGTGTTGGAGTTTTCCTAGACTATGAGGCTTGCACTGTCT
CATTCTTCAATGTCACAAACAATGGATTTCTCATCTATAAGTTTTCTAACTGTCAT
TTTTGTTATCCTGTATTTCCATATTTCAGTCCTATGACATGTGAATTACCCATGAC
TCTGTGCTCACCAAGCTCTTGAACTATCTTAAATACTCAGCCGCTTCTTACCCAGG
TGCATCTCATACACCTGAACCTTCAT

[0045] SEQ ID NO:3 (AY646200) - OMK CypA cDNA

[0046] CCTTGTAGCATCAGCCATGGTCAATCCTACCGTGTTCTTCGACATTGCCGT
CGATGGCGAGCCCTTGGGCCGCGTCTCCTTCGAGCTGTTTGCAGACAAGGTTCCA
AAGACAGCAGAAAACTTTCGTGCTCTGAGCACTGGAGAGAAAGGATTTGGTTAT
AAGGGTTCCTGCTTTCACAGAATTATTCCAGGGTTTATGTGTCAGGGTGGTGACT
TCACACGCCATAATGGCACTGGTGGCAAGTCCATCTACGGGGAGAAATTTGATG
ACGAGAACTTCATCCTAAAGCATACAGGTCCCGGTATCTTGTCCATGGCAAATGC
TGGACCCAACACAAACGGTTCCCAGTTTTTCATCTGCACTGTCAAGACTGAGTGG
TTGGATGGCAAGCATGTGGTCTTTGGCAAGGTGAAAGAAGGCATGAATATTGTG
GAGGCCATGGAGCGCTTTGGGTCCAGGAATGGCAAGACCAGCAAGAAGATCACC
ATTGCTGACTGTGGACAACTTTAATAAGTTTGACTTGTGTTTTGTCTTCACCACCA

GACCATTCCTTCTGTAGCTCAGGAGAGCACCCCTCCACCCCATTTGCTCGCAGTA
TCCTAAAATCTGTGCTCTCGCTGCAGTTCCCTTTGGGTCCCATGTTTTCCTTGTTCC
CTTCCATGCCTAGCTGGATTGCAGAGTTGAGTTAAGTTTATGATTATGAAATAAA
GACTAAATAACAAAAAAAAAAAAAAA

[0047] SEQ ID NO:4 – amino acid sequence of TRIMcyp (Fig. 2A)

[0048] MASRILVNIKEEVTCPICLELLTEPLSLDCGHSFCQACITANHKKSMPHQGERS
CPLCRISYSSENLRPNRHLVNIVERLREVMLSPEEGQKVDHCAHHGEKLVLFCQQDG
NVICWLCERSQEHRGHQTFLVEEVAQKYREKLQVALEMMRQKQKDAEKLEADVRE
EQASWKIQIQNDKTNIMAEFKKRRDILDCEESKELQNLEKEEKNILKRLVQSENDMV
LQTQSVRVLISDLEHRLQGSVMELLQGVDGVIKRIEKVTLQNPKTFLNEKRRIFQTPD
LKGTLQVFKEPTEVQRYWDAAAWDLVASAMVNPTVFFDIAVDGEPLGRVSFELFAD
KVPKTAENFRALSTGEKGFGYKGSCFHRIIPGFMCQGGDFTRHNGTGGKSIYGEKFD
DENFILKHTGPGILSMANAGPNTNGSQFFICTAKTEWLDGKHVVFGKVKEGMNVVE
AMERFGCRYGKTSKKITIADCGQL

[0049] The invention provides for a newly discovered and isolated nucleic acid or polynucleotide that was isolated from an owl monkey, that comprises the nucleotide sequence of SEQ ID NO:1, 2 or 3, or encodes the polypeptide of SEQ ID NO:4. The polypeptide is a fusion between TRIM and cyclophilin, i.e., TRIMcyp, which acts as an anti-HIV-1 factor.

[0050] The invention also provides for nucleic acid variants of any of SEQ ID NO: 1, 2, or 3 having at least about 50% identity to the SEQ ID NO, and encoding a polypeptide having both TRIM activity and cyclophilin activity. The variants may have at least about 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity to the SEQ ID NO. Techniques for determining sequence identity are well known to one skilled in the art, and include, for example, analysis with a sequence comparison algorithm or FASTA version 3.0t78 using default parameters.

[0051] The invention also provides for an isolated nucleic acid which comprises consecutive nucleotides having a sequence complementary to the nucleic acids comprising the nucleotide sequence of SEQ ID NO:1, 2, or 3, or variants of at least about 50% identity thereof. The invention also provides for an isolated nucleic acid encoding a polypeptide comprising consecutive amino acids having a sequence comprising SEQ ID NO:4. Also provided by the

invention is an isolated nucleic acid that hybridizes to a nucleic acid of the invention under conditions of high stringency, moderate stringency, or low stringency.

[0052] The invention further provides for an isolated polypeptide encoded by an isolated nucleic acid of the invention. Purified polypeptides substantially identical to the isolated polypeptide, as determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 using default parameters, are also included in the invention.

[0053] The present invention also provides for methods of making cells resistant to HIV-1 infection. The present invention provides for an isolated fusion protein between TRIM5 and cyclophilin A which was discovered and isolated from Owl monkey, which is a species of primate from South America that are resistant to HIV-1 infection. This newly discovered TRIM-cyclophilin fusion protein provides the Owl monkey with its resistance to HIV. The cyclophilin A part of the fusion molecule specifically binds to the HIV-1 capsid protein once HIV-1 enters the cell. The TRIM5 part of the fusion protein acts as TRIM5 alone has been characterized, causing the degradation of the substance bound to the cyclophilin A portion of the fusion protein, namely the degradation of HIV-1. Thus, the fusion protein has coupled a specific binding tropism, i.e., the binding of cyclophilin to capsid of HIV-1, with the degradation activity of TRIM5, to create a sort of Trojan horse.

[0054] The cyclophilin part of the fusion protein is highly conserved among different species. Cyclophilin is a protein that is present in yeast and in humans. It plays a role in protein-folding and in the biological response to the immunosuppressive drug cyclosporine. The TRIM5 part of the fusion protein is highly variable from one species to another.

[0055] The invention provides a TRIM-cyclophilin fusion protein encoded by the amino acid sequence of SEQ ID NO:4. The invention also provides a TRIM-cyclophilin fusion protein encoded by an isolated polynucleotide of the invention. The isolated polynucleotide may encode a polypeptide comprising, consisting essentially of, or comprising a sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical to, SEQ ID NO:4, and wherein the polypeptide has the function of TRIM and specifically binds a capsid protein of HIV.

[0056] The invention further provides for a purified antibody that specifically binds to a polypeptide of the invention or to a protein encoded by a polynucleotide of the invention. The antibody may be a polyclonal antibody, a monoclonal antibody, or a chimeric antibody.

Polyclonal antibodies may be obtained by procedures which are well known to the skilled artisan, including injecting purified fusion protein into various animals and isolating the antibodies produced in the blood serum. The antibodies may be monoclonal antibodies whose method of production is well known to the art, including, for example, injecting purified fusion protein into a mouse, isolating the spleen cells producing the anti-serum, fusing the cells with tumor cells to form hybridomas and screening the hybridomas.

[0057] Methods for producing the polypeptides of the invention include introducing a nucleic acid encoding the polypeptide into a host cell under conditions that permit expression of the polypeptide by the host cell, and recovering the peptide. A nucleic acid may be introduced into a host cell, for example, with a replicable nucleic acid vector, such as a viral vector, a retroviral vector, an adenovirus vector, or an adeno-associated viral (AAV) vector. Provided for in the invention is a replicable nucleic acid vector comprising a nucleic acid of the invention, and a host organism comprising the replicable nucleic acid vector. Suitable host organisms include a prokaryote, a eukaryote, or a fungus.

[0058] The invention provides for a host cell comprising the recombinant expression construct encoding the TRIMCyp fusion protein as described herein. In another embodiment of the invention, the host cell is stably transformed with the recombinant expression construct described herein. In one embodiment the host cell is a bone marrow cell of a subject. In another embodiment of the invention, the cell is an immortalized cell.

[0059] A nucleic acid, a polypeptide, or a nucleic acid vector of the invention may be used to prepare pharmaceutical compositions of the invention. Methods for preparing a pharmaceutical composition include admixing a polypeptide of the invention or a fragment thereof.

[0060] The pharmaceutical composition further comprises a pharmaceutically acceptable carrier. The carrier comprises a diluent. The carrier may also comprise an appropriate adjuvant, a herpes virus, an adenovirus, a liposome, a microencapsule, a polymer encapsulated cell or a retroviral vector. The pharmaceutically acceptable carrier may be an aerosol, intravenous, oral or topical carrier.

[0061] A nucleic acid, a polypeptide, a nucleic acid vector, or a pharmaceutical composition of the invention is suitable for treating a subject suffering from a disease or condition, such as a retroviral infection. In one embodiment, the invention provides for methods of preventing

retroviral infection in a subject, or for treating a subject with a retroviral infection, by administering to the subject a pharmaceutical composition of the invention. In another embodiment, the invention provides for methods of reducing viral burden, or load, in a subject infected by a virus by administration of a nucleic acid, a polypeptide, or a nucleic acid vector of the invention, to the subject.

[0062] The invention provides for methods of gene therapy of subjects for the prevention or treatment of viral infection comprising administering to the subject an effective amount of a pharmaceutical composition comprising, or consisting essentially of, a polynucleotide encoding a fusion protein having cyclophilin fused to a TRIM polypeptide, wherein the subject's cells takes up the polynucleotide or polypeptide such that the fusion protein is present in cells of the subject, so as to prevent or treat viral infection in the subject. In one embodiment, the treated subject has a reduced viral load, or burden. In one embodiment of this invention, the administering is via an autologous bone marrow transplant to the subject, where the polynucleotides coding for the fusion protein is transfected into the bone marrow cells *ex vivo* and then replaced into the subject. In this case, the bone marrow cells returned to the subject will have taken up (been transduced) the nucleic acid encoding the fusion protein.

[0063] Viral infections that may be treated or prevented by introducing a TRIM-Cyp fusion polypeptide into the subject's cells include an HIV infection, a human T-cell lymphotrophic virus (HTLV) infection, a pox virus infection, a retrovirus infection, a malaria infection, a hepatitis C virus infection, a hepatitis B virus infection, or a vaccinia virus infection. In one embodiment, the pox virus is small pox.

[0064] The present invention provides for the TRIMCyp fusion protein which combines a specific binding activity (specific for HIV-1 capsid protein) and a killing activity (TRIM activity, like ubiquitin). In this way, the HIV-1 is degraded in the cell.

[0065] The invention provides methods for treating HIV infection or preventing HIV infection of a subject by introducing a TRIM-Cyp fusion polypeptide into the subject's cells. The TRIM-Cyp polypeptide binds to a capsid protein of the HIV and subsequently degrades the HIV, thereby treating or preventing the HIV infection in the subject. Resistance to HIV may also be imparted to a subject or to the cells of a subject by administering to the cells of the subject a nucleic acid, a polypeptide, or a nucleic acid vector of the invention.

[0066] The invention also provides for a peptidomimetic comprising an amino acid sequence substantially identical to the amino acid sequence of a polypeptide of the invention. As used herein a "peptidomimetic" refers to a chemical compound that mimics the biological activity of a peptide. In one embodiment of the invention, a peptidomimetic comprises TRIM function and cyclophilin function.

[0067] A peptidomimetic, a nucleic acid, or a polypeptide of the invention is suitable for preparing a therapeutic composition of the invention. The therapeutic composition further comprises a therapeutically acceptable carrier. The carrier may comprise a vector, a liposome, or a viral vector.

[0068] The invention further provides a stem cell comprising a nucleic acid of the invention, wherein the nucleic acid is part of the genome of the stem cell. As used herein a "stem cell" refers to an undifferentiated cell in the bone marrow that has the ability both to multiply and to differentiate into a specific, specialized cell, such as a blood cell. In one embodiment, human hematopoietic stem cells are mobilized from the bone marrow of an HIV-1 infected subject, the cells are transduced *in vitro* with vectors expressing TRIMCyp, and the cells are injected back into the patient as an auto-transplant.

[0069] Also provided in the invention is a method for reducing transposition events in a genome of a cell. The method comprises introducing into the cell a polynucleotide encoding a TRIM-cyclophilin A fusion protein, or the protein encoded by the polynucleotide, so as to inhibit LINE 1 (L1) elements and reduce transposition elements in the cell. As used herein a "LINE" refers to a Long Interspersed Element. Functional L1 elements are about 6,500 bp in length and encode three proteins, including an endonuclease that cuts DNA and a reverse transcriptase that makes a DNA copy of an RNA transcript. L1 activity proceeds as follows: RNA polymerase II transcribes the L1 DNA into RNA; the RNA is translated by ribosomes in the cytoplasm into the proteins; the proteins and RNA join together and reenter the nucleus; the endonuclease cuts a strand of "target" DNA, often in the intron of a gene; and the reverse transcriptase copies the L1 RNA into L1 DNA which is inserted into the target DNA forming a new L1 element there.

[0070] For the purposes of this invention, "administration" means any of the standard methods of administering a pharmaceutical composition known to those skilled in the art. Examples include, but are not limited to, intravenous, intraperitoneal or intramuscular

administration, *in vitro* gene therapy via adenoviral vector or other vector (liposome), *ex vivo* gene therapy, oral, and inhalation. In another embodiment of the invention, the administering is carried out via injection, oral administration, or topical administration. In one embodiment of this invention, the subject is a mammal, e.g., a mouse or a human. Preferably, the mammal is a human. In another embodiment of the invention, the "introducing" or administering is carried out by a means selected from the group consisting of transduction, viral-mediated introduction, adenovirus infection, liposome-mediated transfer, topical application to the cell, and microinjection. In another embodiment of the invention, the carrier is an aqueous carrier, a liposome, a vector, a viral vector, or a lipid carrier.

[0071] Provided by the invention is a topical composition for prevention or treatment of a viral infection which comprises a nucleic acid or a polypeptide of the invention. The topical composition further comprises a topical carrier, and may be used on the skin, in the vagina, in the nose, in the mouth, or on any skin or mucosal surface.

[0072] As used herein "nucleic acid molecule" includes both DNA and RNA and, unless otherwise specified, includes both double-stranded and single-stranded nucleic acids. Also included are hybrids such as DNA-RNA hybrids. Reference to a nucleic acid sequence can also include modified bases as long as the modification does not significantly interfere either with binding of a ligand such as a protein by the nucleic acid or Watson-Crick base pairing.

[0073] Two DNA or polypeptide sequences are "substantially homologous" when at least about 80% (preferably at least about 90%, and most preferably at least about 95%) of the nucleotides or amino acids match over a defined length of the molecule. As used herein, "substantially homologous" also refers to sequences showing identity to the specified DNA or polypeptide sequence. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Laboratory, 2nd ed., Cold Springs Harbor, New York (1989).

[0074] A DNA "coding sequence" or a "nucleotide sequence encoding" a particular protein, is a DNA sequence which is transcribed and translated into a polypeptide *in vivo* or *in vitro* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5'-(amino) terminus and a translation

stop codon at the 3'-(carboxy) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) sources, viral RNA or DNA, and even synthetic nucleotide sequences. A transcription termination sequence will usually be located 3' to the coding sequence.

[0075] "Operably linked" refers to an arrangement of nucleotide sequence elements wherein the components so described are configured so as to perform their usual function. Thus, control sequences operably linked to a coding sequence are capable of effecting the expression of the coding sequence. The control sequences need not be contiguous with the coding sequence, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

[0076] A cell has been "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element, such as a plasmid. In eukaryotic cells, a stably transformed cell is generally one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication, or one which includes stably maintained extrachromosomal plasmids. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the exogenous DNA.

[0077] The present invention encompasses use of virus based vectors for transformation or transfection of the TRIMCyp coding region into a cell. In the case of eukaryotic cells, retrovirus or adenovirus based vectors are preferred. Such vectors contain all or a part of a viral genome, such as long term repeats ("LTRs"), promoters (e.g., CMV promoters, SV40 promoter, RSV promoter), enhancers, and so forth. When the host cell is a prokaryote, bacterial viruses or phages are preferred. Exemplary of such vectors are vectors based upon, e.g., lambda phage. In any case, the vector may comprise elements of more than one virus. The resulting vectors are transfected or transformed into a host cell, which may be eukaryotic or prokaryotic. The gene transfer vector of the present invention may additionally comprise a

gene encoding a marker or reporter molecule to more easily trace expression of the vector. The gene transfer vector may contain more than one gene encoding the same or different foreign polypeptides or RNAs. The gene transfer vector may be any construct which is able to replicate within a host cell and includes plasmids, DNA viruses, retroviruses, as well as isolated nucleotide molecules. Liposome-mediated transfer of the gene transfer vector may also be carried out in the present invention.

[0078] Adenoviruses can be used for transformation or transfection of the nucleic acids of the present invention into cells. Examples of such adenovirus serotypes which can be employed in the present invention are well-known in the art and include more than 40 different human adenoviruses, e.g., Ad12 (subgenus A), Ad3 and Ad7 (Subgenus B), Ad2 and Ad5 (Subgenus C), Ad8 (Subgenus D), Ad4 (Subgenus E), Ad40 (Subgenus F) (Wigand et al, in: Adenovirus DNA, Doerfler, Ed., Martinus Nijhoff Publishing, Boston, pp. 408-441 (1986)). Ad5 of subgroup C is the preferred adenovirus employed in the present invention. This is because Ad5 is a human adenovirus about which a great deal of biochemical and genetic information is known, and it has historically been used for most constructions employing adenovirus as a vector. Also, adenoviral vectors are commercially available, e.g., pCA3 (Microbix Biosystems Inc.). Methods for producing adenovirus vectors are well-known in the art (Berkner et al, Nucleic Acids Res., 11:6003-6020 (1983); van Doren et al, Mol. Cell. Biol., 4:1653-1656 (1984); Ghosh-Choudhury et al, Biochem. Biophys. Res. Commun., 147:964-973 (1987); McGrory et al, Virol., 163:614-617 (1988); and Gluzman et al, in: Eurkaryotic Viral Vectors, Ed. Gluzman, Y. pages 187-192, Cold Spring Harbor Laboratory (1982)). Vectors which can be used in the methods of the present invention include adenoviruses, retroviral vectors, and adeno-associated viral (AAV) vectors. Other virus vectors that may be used for gene transfer into cells include retroviruses such as Moloney murine leukemia virus (MoMuLV); papovaviruses such as JC, SV40, polyoma, adenoviruses; Epstein-Barr Virus (EBV); papilloma viruses, e.g. bovine papilloma virus type I (BPV); vaccinia and poliovirus and other human and animal viruses. Expression can be amplified by placing an amplifiable gene, such as the mouse dihydrofolate reductase (dhfr) gene adjacent to the coding sequence. Cells can then be selected for methotrexate resistance in dhfr-deficient cells. See, e.g. Urlaub et al. (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220; Rungold et al. (1981) J. Mol. and Appl. Genet. 1:165-175.

[0079] It may also be desirable to produce mutants or analogs of the proteins of interest. Mutants or analogs may be prepared by the deletion of a portion of the sequence encoding the protein, by insertion of a sequence, and/or by substitution of one or more nucleotides within the sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, are well known to those skilled in the art. See, e.g., Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Laboratory, 2nd ed., Cold Springs Harbor, New York (1989).

[0080] The constructs can also be used in gene therapy or nucleic acid immunization, to direct the production of the desired gene product *in vivo*, by administering the expression constructs directly to a subject for the *in vivo* translation thereof. See, e.g. EPA Publication No. 336,523 (Dreano et al., published Oct. 11, 1989). Alternatively, gene transfer can be accomplished by transfecting the subject's cells or tissues with the expression constructs *ex vivo* and reintroducing the transformed material into the host. The constructs can be directly introduced into the host organism, i.e., by injection (see International Publication No. WO/90/11092; and Wolff et al., (1990) Science 247:1465-1468). Liposome-mediated gene transfer can also be accomplished using known methods. See, e.g., Hazinski et al., (1991) Am. J. Respir. Cell Mol. Biol. 4:206-209; Brigham et al. (1989) Am. J. Med. Sci. 298:278-281; Canonico et al. (1991) Clin. Res. 39:219A; and Nabel et al. (1990) Science 249:1285-1288. Targeting agents, such as antibodies directed against surface antigens expressed on specific cell types, can be covalently conjugated to the liposomal surface so that the nucleic acid can be delivered to specific tissues and cells for local administration.

[0081] There are several protocols for human gene therapy which have been approved for use by the Recombinant DNA Advisory Committee (RAC) which conform to a general protocol of target cell infection and administration of transfected cells (see for example, Blaese, R.M., et al., 1990; Anderson, W. F., 1992; Culver, K.W. et al., 1991). In addition, U.S. Patent No. 5,399,346 (Anderson, W. F. et al., March 21, 1995, U.S. Serial No. 220,175) describes procedures for retroviral gene transfer. The contents of these support references are incorporated in their entirety into the subject application. Retroviral-mediated gene transfer requires target cells which are undergoing cell division in order to achieve stable integration hence, cells are collected from a subject often by removing blood or bone marrow. It may be necessary to select for a particular subpopulation of the originally harvested cells for use in the infection protocol. Then, a retroviral vector containing the gene(s) of interest would be

mixed into the culture medium. The vector binds to the surface of the subject's cells, enters the cells and inserts the gene of interest randomly into a chromosome. The gene of interest is now stably integrated and will remain in place and be passed to all of the daughter cells as the cells grow in number. The cells may be expanded in culture for a total of 9-10 days before reinfusion (Culver et al., 1991). As the length of time the target cells are left in culture increases, the possibility of contamination also increases, therefore a shorter protocol would be more beneficial.

[0082] One way to get DNA into a target cell is to put it inside a membrane bound sac or vesicle such as a spheroplast or liposome, or by calcium phosphate precipitation ($CaPO_4$) (Graham F. and Van der Eb, A., Virology 52:456 1973; Schaefer-Ridder M., et al., Liposomes as gene carriers: Efficient transduction of mouse L cells by thymidine kinase gene. Science 1982; 215:166; Stavridis J. C., et al., Construction of transferrin-coated liposomes for *in vivo* transport of exogenous DNA to bone marrow erythroblasts in rabbits. Exp Cell Res 1986; 164:568-572).

[0083] A vesicle can be constructed in such a way that its membrane will fuse with the outer membrane of a target cell. The vector of the invention in vesicles can home into the cancer cells. The spheroplasts are maintained in high ionic strength buffer until they can be fused through the mammalian target cell using fusogens such as polyethylene glycol.

[0084] Liposomes are artificial phospholipid vesicles. Vesicles range in size from 0.2 to 4.0 micrometers and can entrap 10% to 40% of an aqueous buffer containing macromolecules. The liposomes protect the DNA from nucleases and facilitate its introduction into target cells. Transfection can also occur through electroporation. Before administration, the modified vectors are suspended in complete PBS at a selected density for injection. In addition to PBS, any osmotically balanced solution which is physiologically compatible with the subject may be used to suspend and inject the modified vectors into the host.

[0085] For injection, the cell suspension is drawn up into the syringe and administered to anesthetized recipients. Multiple injections may be made using this procedure. The viral suspension procedure thus permits administration of genetically modified vectors to any predetermined site in the skin, is relatively non-traumatic, and allows multiple administrations simultaneously in several different sites or the same site using the same viral suspension. Multiple injections may consist of a mixture of therapeutic genes.

[0086] As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise.

[0087] This invention is illustrated in the Example sections that follow. These sections are set forth to aid in an understanding of the invention but are not intended to, and should not be construed to, limit in any way the invention as set forth in the claims which follow thereafter.

[0088] Example 1 - Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1

[0089] Here we show that knockdown of owl monkey CypA by RNA interference (RNAi) correlates with suppression of anti-HIV-1 activity. However, reintroduction of CypA protein to RNAi-treated cells did not restore antiviral activity. A search for additional RNAi targets unearthed *TRIMCyp*, an RNAi-responsive messenger RNA encoding a TRIM5–CypA fusion protein. *TRIMCyp* accounts for post-entry restriction of HIV-1 in owl monkeys and blocks HIV-1 infection when transferred to otherwise infectable human or rat cells. It seems that *TRIMCyp* arose after the divergence of New and Old World primates when a LINE-1 retrotransposon catalysed the insertion of a *CypA* complementary DNA into the *TRIM5* locus. This is the first vertebrate example of a chimaeric gene generated by this mechanism of exon shuffling.

[0090] Post-entry restriction of HIV-1 infection is common among Old World monkeys, but owl monkeys are unique among New World primates in exhibiting this phenotype (1). *Aotus trivirgatus* owl monkey kidney cells (OMK) restrict HIV-1 infection, but are permissive for simian immunodeficiency virus (SIV) infection (1). HIV-1 restriction in OMK cells is completely abrogated when the interaction between HIV-1 capsid and the cellular protein cyclophilin A (CypA) is disrupted (4), either by mutations altering capsid or by treatment of target cells with the cyclophilin-binding drug cyclosporine (CsA). This phenotype is the opposite of that seen in most human cells where the capsid–CypA interaction is required for efficient HIV-1 replication (4, 6-9).

[0091] The paradoxical response to CsA in OMK cells was investigated further using two other drugs: MeIle^4-CsA, a non-immunosuppressive analogue (9), and sanglifehrin, a structurally unrelated compound that also binds cyclophilin (10). As with CsA, treatment of target cells with these compounds permits HIV-1 to infect OMK cells at an efficiency similar to that of SIV (Fig. 1a). Peripheral blood mononuclear cells (PBMC) from a different owl

monkey species, *Aotus nancymaae*, show the same restriction phenotype with respect to SIV, HIV-1 and CsA (Fig. 1b). Identical results were obtained with PBMC from a second animal.

[0092] CsA, MeIle^4-CsA and sanglifehrin bind cyclophilin family members, but not exclusively CypA. To examine the specific role of CypA, we generated stable OMK cell lines with CypA knockdown by RNAi. Of six CypA-specific small hairpin RNAs (shRNAs), three decreased CypA expression (Fig. 1c). Those shRNA constructs that decreased CypA expression abrogated HIV-1 restriction to a corresponding degree (Fig. 1d).

[0093] To determine whether disruption of HIV-1 restriction was due to CypA knockdown, the OMK knockdown cell line with the largest decrease in CypA expression and HIV-1 restriction ($OMK_{MH\text{-}CypA\text{-}147}$) was transfected with non-targetable *CypA* cDNAs (*ntCypA*) bearing silent mutations to make them resistant to the RNAi. A plasmid encoding cell surface H-$2K^K$ was cotransfected so that transfected cells could be enriched using antibodies conjugated to magnetic particles. Although cells selected in this manner were fully restored for CypA expression, they remained deficient for HIV-1 restriction (Fig. 1e). We attempted to restore CypA expression in $OMK_{MH\text{-}CypA\text{-}147}$ cells several times using different methods, including retroviral transduction of *ntCypA* cDNA. In all cases, reconstitution of CypA expression did not restore HIV-1 restriction activity.

[0094] Off-target spread of RNAi has been reported (11), but this seemed an unlikely explanation for our inability to restore restriction to $OMK_{MH\text{-}CypA\text{-}147}$ because CypA knockdown with three different RNAi target sequences was associated with loss of restriction. A more plausible explanation seemed to be knockdown of an unknown mRNA homologous to *CypA*. Multiple screens of OMK cDNA by hybridization or polymerase chain reaction (PCR) yielded only bona fide *CypA* cDNA. Northern analysis was performed to look for transcripts other than *CypA* that were decreased by RNAi in these cells. Total cytoplasmic RNA from $OMK_{MH\text{-}CypA\text{-}147}$, or from control OMK cells treated with RNAi specific for luciferase, was hybridized with *CypA* coding sequence (Fig. 1f). The dominant *CypA* transcript (about 750 nucleotides) was significantly reduced in $OMK_{MH\text{-}CypA\text{-}147}$. Another transcript of approximately 2 kb hybridized to the *CypA* probe and was also decreased by RNAi against CypA.

[0095] To identify the larger transcript detected by northern blot, a size-selected OMK cDNA library (1,700 to 2,500 base pairs, bp) was screened by colony hybridization with a *CypA*

probe. Inserts from four positive colonies were sequenced. Two contained in-frame fusions between *TRIM5* (exons 1 to 7) and a complete *CypA* cDNA. This *TRIMCyp* cDNA was predicted to encode a 54-kD protein chimaera consisting of the amino-terminal 299 amino acids of TRIM5 and the 165-amino-acid CypA protein linked by 11 amino acids encoded by the *CypA* 5′ untranslated region (UTR) (Fig. 2a). 293T cells transfected with a *TRIMCyp* cDNA expression vector produced a protein of the expected size that was reactive with both anti-TRIM5 and anti-CypA antibodies (Fig. 2b).

[0096] *TRIMCyp* mRNA was detectable in OMK cells and in PBMC from two *Aotus nancymaae* monkeys (Fig. 2c, d), but not in the human cell lines Jurkat or HeLa, or in rhesus macaque FRhK4 cells (Fig. 2c). Quantitative reverse transcription (RT)–PCR was used to demonstrate that *TRIMCyp* expression in the CypA knockdown cells used in Fig. 1e, f was decreased tenfold (Fig. 2e).

[0097] To determine whether *TRIMCyp* was sufficient to restore HIV-1 restriction to CypA knockdown OMK cells, $OMK_{MH\text{-}CypA\text{-}147}$ were transduced with a pMIG retroviral vector delivering a *TRIMCyp* cDNA bearing silent mutations that render it resistant to RNAi (*ntTRIMCyp*). pMIG expresses cDNAs fused to an internal ribosome entry site (IRES)–green fluorescent protein (GFP) cassette, permitting identification of transduced cells by GFP expression. pMIG-transduced $OMK_{MH\text{-}CypA\text{-}147}$ cells were assayed for their ability to restrict an HIV-1 vector that delivers the DsRed fluorescent protein. $OMK_{MH\text{-}CypA\text{-}147}$ cells transduced with pMIG-*ntTRIMCyp* restricted HIV-1, while those transduced with empty vector, or vector delivering $ntCypA_{OM}$, did not (Fig. 2f). Loss of restriction in OMK cells caused by RNAi against CypA (Fig. 1c, d) could therefore be attributed to *TRIMCyp* knockdown.

[0098] To see if transfer of *TRIMCyp* confers post-entry restriction of HIV-1 to cells derived from other species, we generated stable human TE671 cell lines expressing *TRIMCyp*, $CypA_{OM}$, or *TRIMStop*, a construct in which *TRIMCyp* has been truncated with a stop codon replacing the first codon of the CypA domain. TE671 cells expressing *TRIMCyp* were highly resistant to HIV-1 infection, while expression of *TRIMStop* or $CypA_{OM}$ had no effect on HIV-1 titre (Fig. 3a). As expected, SIV_{MAC239} infection of TE671 cells was unaffected by *TRIMCyp* (Fig. 3b). HIV-1 restriction mediated by TRIMCyp was bypassed by treating target cells with CsA during infection, or by infection with HIV-1 bearing the G89V capsid mutation that prevents CypA binding (Fig. 3c). HeLa cells transduced with pMIG-*TRIMCyp*

exhibited the same restriction (Fig. 3d). These data demonstrate that HIV-1 restriction by TRIMCyp is capsid-specific and cell-type independent. The CypA domain of TRIMCyp is essential for restriction and disruption of CypA–capsid binding abrogates TRIMCyp-mediated restriction.

[0099] Sequencing of OMK genomic DNA, and comparison with human sequence, revealed a complete *CypA* cDNA between *TRIM5* exons 7 and 8 (Fig. 4a). We did not detect an owl monkey *TRIM5* allele lacking the *CypA* insertion. The inserted *CypA* cDNA is 95% identical to the bona fide owl monkey *CypA* cDNA, but only 88% identical to human or rhesus macaque *CypA*, suggesting that *CypA* insertion into *TRIM5* occurred after the divergence of New and Old World primates. Because other New World monkeys lack post-entry HIV-1 restriction activity (1), the *CypA* insertion appears to be unique to owl monkeys.

[00100] The *CypA* insertion bears the hallmarks of LINE-1 (L1)-mediated retrotransposition (12). These include flanking 16-bp direct repeats consistent with target site duplication, a processed cDNA devoid of introns and accompanied by a poly-A tail, and insertion at a consensus, A-rich L1 endonuclease recognition site (13) (Fig. 4b, c). Mammalian genomes possess many reverse-transcribed *CypA* pseudogenes which, by definition, are defective (14). *TRIMCyp* is unusual in that a complete, processed *CypA* cDNA has generated a new *CypA* exon that is expressed and spliced to *TRIM5* exon 7 to encode a novel chimaeric protein.

[00101] TRIMCyp's mechanism of action may be hinted at by its structure and by recent experiments with TRIM5 homologues from other species. The TRIM5 ring-finger domain confers E3 ubiquitin ligase activity (15), suggesting that TRIMCyp attaches ubiquitin to incoming HIV-1 virion proteins. Human cells possess a TRIM5 homologue and HIV-1 infection of these cells is stimulated by proteasome inhibitors (16). We were unable to detect an effect of these drugs on HIV-1 restriction of OMK cells, although these cells are resistant to other drugs, including antibiotics commonly used for selection of mammalian clones. TRIM5 possesses a coiled-coil domain which causes the protein to aggregate and form cytoplasmic bodies (15, 17). TRIMCyp forms similar structures and associates with human TRIM5-α in the yeast two-hybrid system. Though TRIMCyp is functional in non-primate cells (rat fibroblasts, Fig. 3e), restriction may require recruitment of other TRIM family members.

[00102] The TRIMCyp carboxy terminus, which is required for restriction (Fig. 3a, d), bears a complete CypA protein that probably functions autonomously to recognize HIV-1 capsid (6). This would not be surprising because cyclophilins frequently exist as discrete domains within complex proteins (18). In rhesus macaques, TRIM5-α accounts for a potent HIV-1 restriction, and the C-terminal B30.2 (SPRY) domain that distinguishes the α-isoform of TRIM5 is essential for this activity (5). RT–PCR with primers based on the owl monkey genomic sequence failed to detect an owl monkey *TRIM5-α* transcript (one containing the exon 8-encoded B30.2 domain instead of the CypA domain) (Fig. 2c). Because the CypA domain of TRIMCyp substitutes for the B30.2 domain present in macaque TRIM5-α, it is likely that the C terminus of TRIM5-α is also responsible for recognition of incoming viral capsids.

[00103] Since the discovery of transposons over 50 years ago (19), it has become clear that these elements play a fundamental role in evolution. L1 elements are the most abundant autonomous retrotransposons in the human genome and are believed to function *in trans* to retrotranspose *Alu* elements and processed pseudogenes (20-22). Consistent with the exon shuffling model (23), L1 elements have been shown experimentally to transfer non-L1 sequences into existing genes to generate new gene chimaeras (24). Outside the laboratory, a retrotransposed *SVA* element forms the last exon of a leptin receptor (25) and one *ATM* exon retrotransposed into a new genomic location (26). The only previous report of a retrotransposed complete mRNA generating a new exon in a previously existing gene is *jingwei* in *Drosophila melanogaster* (27). *TRIMCyp* is the first example of the genesis of such a chimaeric gene in vertebrates. Interestingly, among primates, owl monkeys have relatively high rates of *Alu* amplification (28), an indication of increased L1-mediated transposition in these animals. The generation of *TRIMCyp* probably represents one example of a process that has played a role in the evolution of many genes.

[00104] **Methods**

[00105] **Plasmids** - Most constructs were previously described (4). CSRW, an HIV-1 vector derived from CSGW, bears DsRed (Clontech). p8.9NΔSB is an HIV-1 packaging vector based on pCMVΔR8.91. pMACS-KK.II (Miltenyi Biotec) encodes truncated murine cell-surface major histocompatibility complex (MHC) I H-2K^{K}.

[00106] Oligonucleotides encoding shRNAs targeting CypA (Table 1, oligos 9–20) or firefly luciferase (oligos 21 and 22) were ligated into pSUPER (29). The shRNA expression cassette was subcloned into pMSCVΔU3 (30) to generate pMH.

[00107] *ntTRIMCyp* was generated by PCR using oligos 24 and 25, and 23 and 26. *TRIMStop* replaces the CypA start codon with a stop codon (oligos 25 and 29). $ntCypA_{OM}$ and $ntCypA_{Hu}$ are CypA cDNAs resistant to pMH–CypA147 that were generated by PCR using oligos 23–27. *ntTRIMCyp*, *TRIMStop* and *ntCypA* were cloned into pcDNA3.1, pMIG and pLPCX.

[00108] **Northern Blots -** Total cytoplasmic RNA (RNeasy, Qiagen) was fractionated by standard protocols using formaldehyde agarose gel electrophoresis, transferred to Hybond-N+ nylon membranes (Amersham), and fixed by ultraviolet crosslinking (Stratalinker, Stratagene). Probes were owl monkey *CypA* or human *CypB* coding sequence, ^{32}P-labelled by random priming (RediPrimeII, Amersham). Blots were hybridized in Rapid-hyb (Amersham), and visualized on a PhosphorImager (Molecular Devices).

[00109] **RT–PCR and real time RT–PCR -** Total RNA from cultured cells or PBMC (RNeasy, Qiagen) was reverse-transcribed into cDNA by random priming (Superscript II, Invitrogen). Primers 1 and 2 were used to amplify *TRIMCyp* cDNA, primers 5 and 6 to amplify *CypA*, and primers 30 and 31 to amplify *TRIM5-α*. Real-time RT–PCR was performed as previously described (30) with product detection using SYBR Green (Molecular Probes). Primers 1 and 2 were used to amplify *TRIMCyp*. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) control curves (primers 7 and 8) were identical between samples in the experiments presented here. Cycling conditions were 40 cycles: 94 °C, 15 s; 55 °C, 15 s; 72 °C, 30 s.

[00110] **cDNA library screen -** OMK cDNA was size-selected (1,700–2,500 bp) by agarose gel electrophoresis and cloned into pBluescriptII-KS. 100,000 colonies were screened by hybridization with a *CypA* probe. OMK *CypA* cDNA was cloned similarly but without size selection.

[00111] **Genomic PCR** – OMK genomic DNA was amplified by PCR using oligos 1 and 2, oligos 3 and 4, or oligos 32 and 33 (Table 1). PCR products were purified (Qiaquick, Qiagen) and sequenced.

[00112] **Cell lines and PBMCs** - OMK, 293T, TE671, HeLa and Rat2 cells were obtained from ATCC. Owl monkey PBMC were isolated with Ficoll-Paque Plus (Pharmacia), stimulated with 2 μg ml^{-1} PHA (Sigma), and cultured in RPMI supplemented with 20% FBS, 20 U ml^{-1} recombinant human IL-2 (Boehringer Mannheim) and 2 mM L-glutamine.

[00113] **Drugs** – CsA (Bedford Laboratories) was prepared in DMSO at 10 mg ml^{-1}. MeIle^4–CsA and sanglifehrin (gifts from Novartis, Basel) were prepared in DMSO at 10 mg ml^{-1} and 10 mM, respectively. All drugs were diluted in tissue culture medium to 2.5 μM and added at the time of infection.

[00114] **Immunoblots** - Whole-cell extracts were fractionated by SDS–polyacrylamide gel electrophoresis (PAGE), transferred to PVDF membranes, and probed using rabbit anti-CypA (Biomol), goat anti-TRIM5 (Abcam), or goat anti-actin (Santa Cruz Biotechnology).

[00115] **Viruses and retroviral vectors** - Vectors and viruses were produced by transfection of 293T cells using lipofectamine 2000 (Invitrogen) and pseudotyped with VSV-G. pMH, pMIG and pLPCX vectors were produced by co-transfection of the vector plasmid, pCL-*Eco* and pMD.G using a 5:5:1 ratio of the three plasmids in each transfection. HIV-DsRed and HIV-GFP vectors were produced in 293T cells by co-transfection of CSGW or CSRW with p8.9NΔSB (wild type or G89V mutant) and pMD.G, also at 5:5:1 ratio. HIV/Env-/GFP and SIV/Env-/GFP viruses were produced by cotransfection of the wild type or G89V HIV/Env-/GFP plasmids with pMD.G at a 10:1 ratio. All vectors and viruses were harvested 48 h post-transfection, filtered (0.45-μm filter, Pall Acrodisc), aliquoted, and stored at –80 °C. Stocks were normalized by reverse transcriptase activity or p24 ELISA (8).

[00116] **OMK cell transfection and magnetic selection** - 5×10^5 OMK cells per well in six-well plates were co-transfected with 1 μg pcDNA3.1 expression vector and 0.1 μg pMACS-K^K.II using 6 μl lipofectamine 2000. H-$2K^K$-positive cells were selected (MACSelect KK.II, Miltenyi Biotec) 24 h post-transfection, and either processed for western blot or infected with HIV-GFP.

[00117] **Generation of stable cell lines** - To generate stable RNAi knockdown lines, 1 ml of pMH vector-containing media was pelleted onto OMK cells (1,200*g* for 72 min) in

12-well plates. Infection was repeated 24 h later. *ntTRIMCyp*, *TRIMStop* and *ntCypA$_{OM}$* cDNAs were transduced with either pMIG (OMK and HeLa cells) or pLPCX (TE671 and Rat2 cells). pLPCX-infected cells were selected in 1 μg ml^{-1} puromycin.

[00118] **Infection assays** - Cells were assayed by flow cytometry 48 to 72 h post-infection to determine the percentage of cells that were GFP-positive (FL-1) or DsRed-positive (FL-2). Cells transduced with pMIG vectors and infected with HIV-DsRed were gated to limit the analysis to GFP-positive cells.

Table 1.

	Oligo Name	Oligo Sequence	SEQ ID NO:
1	TRIMCyp781F	5'-GTGACTTTGCAGAATCCAAAAACC-3'	5
2	TRIMCyp963R	5'-GGCAATGTCGAAGAACACAGTAGG-3'	6
3	CypA84 F	5'-GGTTCCAAAGACAGCAGAA-3'	7
4	TRIM1066 R	5'-TTCCCTGATGTGATACTTTG-3'	8
5	CypAStartF	5'-ATGGTCAACCCCACCGTGTT-3'	9
6	CypA147R	5'-TCTGTGAAAGCAGGAACCC-3'	10
7	GAPDHF	5'-CCACATCGCTCAGACACCAT-3'	11
8	GAPDHR	5'-GGCAACAATATCCACTTTACCAGAGT-3'	12
9	sh147sense	5'-GATCCCCGGGTTCCTGCTTTCACAGATTCAAGAGATCTGTGAAAG CAGGAACCCTTTTTGGAAA-3'	13
10	sh147anti	5'-AGCTTTTCCAAAAAGGGTTCCTGCTTTCACAGATCTCTTGAATCTG TGAAAGCAGGAACCCGGG-3'	14
11	sh354sense	5'-GATCCCCGACTGAGTGGTTGGATGGCTTCAAGAGAGCCATCCAAC CACTCAGTCTTTTTGGAAA-3'	15
12	sh354anti	5'-AGCTTTTCCAAAAAGACTGAGTGGTTGGATGGCTCTCTTGAAGCC ATCCAACCACTCAGTCGGG-3'	16
13	sh375sense	5'-GATCCCCGCATGTGGTCTTTGGCAAGGTGTTCAAGAGACACCTTG CCAAAGACCACATGCTTTTTGGAAA-3'	17
14	sh375anti	5'-AGCTTTTCCAAAAAGCATGTGGTCTTTGGCAAGGTGTCTCTTGAA CACCTTGCCAAAGACCACATGCGGG-3'	18
15	sh404sense	5'-GATCCCCGCATGAATATTGTGGAGGCCATGGTTCAAGAGACCATG GCCTCCACAATATTCATGCTTTTTGGAAA-3'	19
16	sh404anti	5'-AGCTTTTCCAAAAAGCATGAATATTGTGGAGGCCATGGTCTCTTG AACCATGGCCTCCACAATATTCATGCGGG-3'	20
17	sh465sense	5'-GATCCCCGATCACCATTGCTGACTGTGTTCAAGAGACACAGTCAG CAATGGTGATCTTTTTGGAAA-3'	21
18	sh465anti	5'-AGCTTTTCCAAAAAGATCACCATTGCTGACTGTGTCTCTTGAACA CAGTCAGCAATGGTGATCGGG-3'	22
19	sh3'UTRsense	5'-GATCCCCTCTGTGCTCTCGCTGCAGTTTCAAGAGAACTGCAGCGA GAGCACAGATTTTTGGAAA-3'	23
20	sh3'UTRanti	5'-AGCTTTTCCAAAAATCTGTGCTCTCGCTGCAGTTCTCTTGAAACTG CAGCGAGAGCACAGAGGG-3'	24
21	shLUCsense	5'-GATCCCCCGTACGCGGAATACTTCGATTCAAGAGATCGAAGTATT CCGCGTACGTTTTTGGAAA-3'	25
22	shLUCanti	5'-AGCTTTTCCAAAAACGTACGCGGAATACTTCGATCTCTTGAATCG AAGTATTCCGCGTACGGGG-3'	26
23	CypNTsense	5'-TTTGGTTATAAAGGCAGCTGTTTCCATAGGATTATTCCA-3'	27
24	CypNTanti	5'-TGGAATAATCCTATGGAAACAGCTGCCTTTATAACCAAA-3'	28
25	CypAStart+Bam	5'-ACGTGGATCCATGGTCAACCCCACCGTGTT-3'	29
26	CypAomkStop+R1	5'-ACGTGAATTCTTATTAGAGTTGTCCACAGTCAGC-3'	30
27	CypAhumStop+R1	5'-ACGTGAATTCTTATTCGAGTTGTCCACAGTCAGC-3'	31
28	TRIMCypStart+Bam	5'-ACGTGGATCCGCCATGGCTTCCAGAATCCTGGTC-3'	32

29	TrimStop+R1	5'-ACGTGAATTCTTATTAGGCTGATGCTACAAGGTCCCA-3'	33

[00119] Example 2 - Disruption of Human TRIM5α Antiviral Activity by Nonhuman Primate Orthologues

[00120] Human immunodeficiency virus type 1 (HIV-1) infection is blocked by the alpha isoform of macaque TRIM5 ($TRIM5\alpha_{rh}$) or by the product of the owl monkey TRIM5-cyclophilin A gene fusion (TRIMCyp). Human TRIM5α potently restricts specific strains of murine leukemia virus (N-MLV) but has only a modest effect on HIV-1. The amino termini of TRIM5 orthologues are highly conserved and possess a coiled-coil domain that promotes homomultimerization. Here we show that heterologous expression of $TRIM5\alpha_{rh}$ or TRIMCyp in human cells interferes with the anti-N-MLV activity of endogenous human TRIM5α ($TRIM5\alpha_{hu}$). Deletion of the cyclophilin domain from TRIMCyp has no effect on heteromultimerization or colocalization with $TRIM5\alpha_{hu}$ but prevents interference with anti-N-MLV activity. These data demonstrate that TRIM5 orthologues form heteromultimers and indicate that C-terminal extensions alter virus recognition by multimers of these proteins.

[00121] TRIM5 proteins inhibit the infectivity of a range of different retroviruses in a species-specific fashion (40). The capsid protein (CA) is the viral determinant for susceptibility to this restriction (33, 44). Rhesus macaque TRIM5α ($TRIM5\alpha_{rh}$) restricts human immunodeficiency virus type 1 (HIV-1) replication (5). Human TRIM5α ($TRIM5\alpha_{hu}$) restricts "N-tropic" strains of the murine leukemia virus (N-MLV) (38, 39, 42, 46). In owl monkey cells, HIV-1 is inhibited by TRIMCyp, the product of the TRIM5-cyclophilin (CypA) gene fusion (reference 41, see also Example 1). The restriction activities of $TRIM5\alpha_{rh}$ and TRIMCyp are conferred to nonrestrictive cells upon transduction of the respective cDNAs (reference 5, see also Example 1). CypA modulates the restriction of HIV-1 in human and owl monkey cells in opposite ways: HIV-1 CA binding to the CypA domain of TRIMCyp (34, 35) is necessary for inhibition of HIV-1 in owl monkey cells, while "free" CypA appears to protect HIV-1 from restriction in human cells (4).

[00122] At the C-terminus of TRIM5α is a variable SPRY domain that determines the species specificity of restriction (38, 39, 46). In owl monkeys, the SPRY domain was replaced by CypA via L1-mediated retrotransposition (reference 41, see also Example 1).

TRIM5α and TRIMCyp both contain a tripartite motif, composed of RING finger, B-Box, and coiled-coil domains (reference 17, see also Example 1), that exhibits E3 ubiquitin ligase activity (5, 36). The coiled-coil domain promotes the formation of TRIM5 homomultimers (17). Here we asked whether TRIM5α or TRIMCyp associates with TRIM5α_{hu} and alters the antiviral activity of the human protein.

[00123] We transduced human rhabdomyosarcoma TE671 cells (45) with previously described LPCX vectors (reference 5, see also Example 1) bearing cDNAs for TRIM5α_{rh}, TRIMCyp, or owl monkey CypA. Cells were also transduced with a vector expressing TRIMStop, a truncated version of TRIMCyp lacking the CypA domain (see Example 1). Pools of transduced cells were selected in puromycin and then assessed for susceptibility to infection with HIV-$1_{NL\text{-}GFP}$ (32). For each transduced population we also tested the effect on HIV-1 infectivity of cyclosporine A (CsA), a drug that competes with HIV-1 CA for binding to CypA (6). We monitored the percentage of infected (GFP-expressing) cells by fluorescence-activated cell sorting (FACS) and the synthesis of viral cDNA in the infected cells using a Southern blot designed to detect full-length, linear viral cDNA and circular viral cDNAs that form in the nucleus (31, 32, 47).

[00124] As expected, both TRIM5α_{rh} and TRIMCyp inhibited HIV-1 infection of TE671 cells and inhibited HIV-1 cDNA synthesis (Fig. 6). TRIMCyp inhibited HIV-1 replication roughly fivefold more efficiently than TRIM5α_{rh}, consistent with the higher levels of HIV-1 restriction in owl monkey cells than in macaque cells (reference 31, see also Example 1). CsA treatment rescued HIV-1 replication in TRIMCyp-expressing cells (see Example 1). CsA also enhanced HIV-1 infection of TE671-TRIM5α_{rh}, indicating that CsA partially countered HIV-1 restriction when TRIM5α_{rh} was expressed in human cells. This result was expected, as CsA also counteracts the restriction to HIV-1 in Old World monkey cells (31). At the high multiplicity of infection used here, CsA had little effect on HIV-1 infectivity; in experiments using a lower multiplicity of infection, CsA modestly decreased HIV-1 infection of control TE671 cells (4).

[00125] Next, we analyzed MLV replication in TE671- TRIM5α_{rh}, TE671-TRIMCyp, and the control TE671-vector cells. We infected these cells with N- or B-tropic, GFP-expressing MLV vectors (32) that had identical titers in nonrestrictive *Mus dunni* tail fibroblasts and were normalized based on infection of these cells (45). In control cells, B-

MLV was ~200-fold more infectious than N-MLV (Fig. 7a). As_2O_3, a drug that counteracts restriction to N-MLV in TE671 cells (32, 39), specifically increased N-MLV infectivity by 10-fold or more (Fig. 7). In cells expressing TRIM5α_{rh}, the N-MLV replication defect was reduced to about 20-fold (Fig. 7a); in cells expressing TRIMCyp, N-tropic restriction was fully abrogated (Fig. 7a). In either case, As_2O_3 no longer enhanced infectivity, as would be expected for cells lacking the antiviral activity targeting N-MLV.

[00126] We also performed the reciprocal experiment, transducing owl monkey OMK cells with TRIM5α_{hu} and challenging them with MLV or HIV-1. TRIM5α_{hu} had only a small (approximately twofold) restrictive effect on the replication of N-MLV and had no effect on B-MLV (Fig. 7b). Restriction of HIV-1 was similar in cells expressing TRIM5α_{hu} to that in the control cells (Fig. 7c), and HIV-1 replication was rescued by CsA in both cell lines, consistent with previous reports (reference 4, see also Example 1). Altogether, the data in Fig. 7 suggest that TRIMCyp is dominant over TRIM5α_{hu}.

[00127] Since TRIMCyp (but not TRIM5α_{rh}) completely suppressed the antiviral activity of TRIM5α_{hu}, we further investigated the effects of this orthologue. TRIMStop, a truncated version of TRIMCyp lacking the CypA domain (see Example 1), did not affect the capacity of TE671 cells to restrict N-MLV replication (Fig. 8a). To determine if this was because TRIMStop was expressed at lower levels than TRIMCyp, we performed reverse transcription-PCR (RT-PCR) using conventional methods (see Example 1). TRIMStop mRNA was expressed at least as well as TRIMCyp mRNA (Fig. 8b); TRIM5α_{hu} was expressed at similar levels in all three cell lines (Fig. 8b). By using Western blotting, we could not detect TRIM5 protein in the TE671 cell lines, but when 293T cells were transfected with the LPCX plasmids, TRIMStop was expressed at higher levels than TRIMCyp (Fig. 8c).

[00128] TRIM5 homomultimerization is promoted by the coiled-coil domain (17), which TRIMCyp also possesses. We hypothesized that the different TRIM5 orthologues heteromultimerize with each other and that TRIMStop did not interfere with TRIM5α_{hu} activity because these particular proteins are incapable of interacting. We used the yeast two-hybrid system to analyze interactions between TRIMStop, TRIMCyp, TRIM5α_{hu}, TRIM5α_{rh}, HIV-1 Gag, and huTRIMStop, a version of TRIM5α_{hu} which, like TRIMCyp, lacks the C-terminal SPRY domain. Fusions of these proteins with LexA and/or B42 were constructed, and the interactions between fusion proteins were analyzed using a previously described

system (37) in which reporter gene β-galactosidase activity was assessed with a quantitative assay (43). TRIMCyp interacted equally well with TRIMCyp, TRIM5α_{rh}, TRIM5α_{hu}, TRIMStop, huTRIMStop, and HIV-1 Gag (Fig. 9a). Though TRIMStop was not able to interact with Gag, presumably because the CypA domain was deleted, this protein was as competent as TRIMCyp in interactions with each of the TRIM5 orthologues.

[00129] To investigate TRIM5:TRIMCyp heterodimerization in mammalian cells, we expressed TRIM5α_{hu} in fusion with glutathione-S-transferase (GST) and transfected 293T cells with either GST or GST-TRIM5α_{hu}. These cells were cotransfected with the LPCX plasmid constructs described above that express TRIMCyp or TRIMStop. GST pull down on glutathione-Sepharose beads (Sigma) followed by Western blotting with anti-TRIM5 antibody showed that both TRIMCyp and TRIMStop associated with TRIM5α_{hu} (Fig. 9b). The blot was also probed with an anti-cyclophilin A antibody, confirming the identity of TRIMCyp (Fig. 9b).

[00130] Finally, to examine the distribution of the tagged proteins in cells by immunofluorescence, we cloned TRIMCyp and TRIMStop into p3xFLAG.CMV (SIGMA), encoding N-terminal FLAG-tagged versions of the two proteins. These constructs were each transfected into TE671 cells and detected with anti-FLAG antibody. Both TRIMCyp and TRIMStop were diffusely distributed in the cytoplasm and concentrated around the nucleus in about half of the cells. TRIM5α_{hu}, expressed in fusion with GST and detected with anti-GST antibody, was cytoplasmic as well and was partly localized to cytoplasmic bodies as previously reported (17). When TRIMCyp or TRIMStop were coexpressed in the same cells with TRIM5α_{hu}, both TRIMCyp and TRIMStop showed partial localization to TRIM5α_{hu} cytoplasmic bodies, suggesting that TRIMCyp and TRIMStop are both capable of heterodimerizing with TRIM5α_{hu} in cells. Altogether, the results shown in Fig. 9 and 10 show that TRIM5 orthologues from different primate species heteromultimerize with each other and that failure of TRIMStop to block restriction activity of TRIM5α_{hu} is not due to failure to multimerize.

[00131] In this Example we show that TRIM5 proteins from different primate species interact with each other and can interfere with each other's function. The CypA domain of TRIMCyp is necessary for interference with endogenous TRIM5α (Fig. 9). CsA did not affect interference by TRIMCyp, demonstrating that CypA peptidyl-isomerase activity was

not relevant for the effect. A likely possibility is that when TRIM5α_{hu} /TRIMCyp heterodimers are formed, the cyclophilin A domain of TRIMCyp interferes with TRIM5α_{hu}'s ability to recognize the N-MLV target. Perhaps TRIM5α and TRIMCyp function as multimers, with the TRIM5 C-terminus facing the viral target (consistent with the role of CypA in binding HIV-1 CA). Similar to the results reported here with different TRIM5 orthologues, the gamma isoform of macaque TRIM5 was found to inhibit TRIM5α_{rh} anti-HIV restriction activity (5).

[00132] Example 3 – TRIM5α selectively binds a restriction-sensitive retroviral capsid

[00133] In most primate species, retroviral restriction requires the C-terminal SPRY domain unique to the α-isoform of TRIM5, but the mechanism by which susceptible viruses are recognized and targeted for restriction is unknown. Here we show that TRIM5α binds retroviral CA from detergent-stripped virions in a SPRY-dependent manner with sufficient discrimination to account for the exquisite specificity of restriction.

[00134] Two independent screens identified TRIM5 as a potent retrovirus restriction element that targets select viruses after entry into primate cells (reference 5, see also Example 1). The biochemical basis for specificity of restriction is only evident in cells of the owl monkey where HIV-1 CA is recognized by the C-terminal cyclophilin domain that is unique to the TRIM5 orthologue found in this genus (reference 41, see also Examples 1 and 2). In all other primates, including humans and macaques, potent CA-specific restriction is conferred by the TRIM5α isoform (5, 38, 39, 42, 46, 48), which possesses a C-terminal SPRY domain (49). The mechanism by which TRIM5α selects retroviruses bearing particular CAs for restriction is unknown, though the TRIM5α SPRY domain is required for restriction and variation in SPRY amino acid residues determines the CA-specificity of given TRIM5α orthologues (48, 50-52).

[00135] Conventional biochemical and two-hybrid experiments failed to detect an interaction between TRIM5α and CA. The observation that non-infectious virus-like particles saturate TRIM5α-mediated restriction (53), but only if the particles bear a mature core from a restriction-sensitive virus (2, 4) suggests that the TRIM5α SPRY domain recognizes a complex structure unique to the core of susceptible virions. Consistent with this

model, expression within target cells of *gag*, *gag-pol*, or *gag* fragments encoding CA, CA-NC, or ubiquitin-CA-NC fusions, failed to block restriction activity.

[00136] Retrovirion cores can be liberated from the viral membrane envelope by detergent (54). HIV-1 virion cores were prepared with several different detergents and mixed with recombinant TRIM5 orthologues. After TRIM5 enrichment by affinity chromatography, CA associated with owl monkey TRIMCypA, as reported with other methods (reference 41, see also Example 2), but not with the equally potent HIV-1 restriction factor rhesus macaque TRIM5α.

[00137] We then selected murine leukemia virus (MLV) for study because, relative to HIV-1, MLV CA remains tightly associated with viral reverse transcription (RT) and preintegration complexes (55, 56). MLV strains bearing an arginine at CA residue 110 (so-called N-MLV) are highly susceptible to restriction by human TRIM5α whereas MLV virions bearing glutamate in this position (B-MLV) are completely resistant to restriction (38, 39, 42, 46).

[00138] VSV G-pseudotyped N- and B-tropic MLV virions were produced as previously described (45) and, after normalization on non-restrictive *Mus dunni* cells, N-MLV was roughly 100-fold less infectious than B-MLV on HeLa cells (Figure 11a). Full-length human TRIM5α was then produced as a GST-fusion protein in 293T cells and mixed with purified N-MLV virions. CAp30, the major MLV core protein constituent, associated with TRIM5α (Figure 11b). CAp30 from B-MLV virions did not associate with TRIM5α (Figure 11b) demonstrating that TRIM5α binding was specific for restriction sensitive CA. CAp30 did not associate with TRIM5 lacking the SPRY domain (Figure 11b), indicating that the SPRY-domain is required for CA-recognition.

[00139] Retroviral restriction specificity thus seems to be determined by TRIM5α binding to CA in a process that requires the SPRY domain. The fact that TRIM5α recognized retroviral CA presented by detergent-stripped virion cores, but not free CA protein, suggests that the SPRY domain recognizes a complex surface of multimerized CA. Once cores of restriction-sensitive viruses are singled out by the SPRY domain, TRIM5α blocks retroviral RT (5) by a mechanism that awaits elucidation. The invention provides for methods of using the TRIMCyp polypeptides for achieving antiviral activity, wherein the antiviral activity of TRIM5α is harnessed to block HIV-1 infection in people.

[00140] Example 4 – Human TRIMCyp

[00141] Human TRIM5 is fused to cyclophilin A to make the human equivalent to the owl monkey TRIM Cyp (Figure 12). The human version restricts HIV-1 and is optimized to make the most potent anti-HIV-1 version of human TRIMCyp.

[00142] From a gene therapy point of view, the human version is more appealing than the owl monkey version because it would be less likely to be recognized by the human immune system as foreign. Expression of a protein that is not normally expressed in a subject in the subject's cells runs the risk of an immune response against the foreign protein, thereby limiting the effectiveness of the gene therapy. Immune responses include allergic reactions, which could be life-threatening. Alternatively, the cells harboring the foreign gene are eliminated, rendering the therapy useless.

[00143] Human hematopoietic stem cells (CD34+ CD38negative) obtained from human placental/umbilical cord blood are transduced using HIV-1-based lentiviral vectors. These cells are then differentiated into T cells and challenged with HIV-1, presenting an effective therapy for blocking HIV-1 infection. Ultimately, human hematopoietic stem cells are mobilized from the bone marrow of HIV-1 infected subjects, the cells are transduced *in vitro* with lentiviral vectors expressing TRIMCyp, and the cells are injected back into the patient as an auto-transplant.

[00144] The following documents are hereby incorporated by reference into this patent application in their entireties for all purposes.

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[00201] TRIMCyp and CypA sequences have been deposited in GenBank (accession numbers AY646198 (SEQ ID NO: 1), AY646199 (SEQ ID NO: 2) and AY646200 (SEQ ID NO: 3)).

[00202] As various changes can be made in the above methods and compositions without departing from the scope and spirit of the invention as described, it is intended that

all subject matter contained in the above description, shown in the accompanying drawings, or defined in the appended claims be interpreted as illustrative, and not in a limiting sense.

What is claimed is:

1. An isolated nucleic acid which comprises consecutive nucleotides having a sequence selected from the group consisting of: SEQ ID NO: 1 (TRIM-Cyp sequence), SEQ ID NO:2 (Aotus TRIM5 Locus (partial genomic sequence)) , SEQ ID NO:3 (OMK CypA cDNA) and a variant of any one of said SEQ ID NOS having at least about 50% identity to the one SEQ ID NO, and encoding a polypeptide having both TRIM activity and cyclophilin activity.

2. An isolated nucleic acid which comprises consecutive nucleotides having a sequence complementary to the nucleic acid of claim 1.

3. The nucleic acid of claim 1, wherein the variant has at least about 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identity to the one SEQ ID NO, as determined by analysis with a sequence comparison algorithm.

4. An isolated nucleic acid that hybridizes to a nucleic acid of claim 1 under conditions of high stringency.

5. An isolated nucleic acid that hybridizes to a nucleic acid of claim 1 under conditions of moderate stringency.

6. An isolated nucleic acid that hybridizes to a nucleic acid of claim 1 under conditions of low stringency.

7. An isolated nucleic acid encoding a polypeptide comprising consecutive amino acids having a sequence comprising SEQ ID NO: 4.

8. An isolated polypeptide encoded by the nucleic acid of any of claims 1 to 7.

9. A purified polypeptide substantially identical to the polypeptide of claim 8 as determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 using default parameters.

10. A polypeptide having the amino acid sequence of SEQ ID NO:4.

11. An isolated polynucleotide encoding a TRIM-cyclophilin fusion protein.

12. The isolated polynucleotide of claim 11, wherein the polynucleotide encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:4.

13. The isolated polynucleotide of claim 11, wherein the polynucleotide encodes a polypeptide consisting essentially of the amino acid sequence of SEQ ID NO:4.

14. The isolated polynucleotide of claim 11, wherein the polynucleotide encodes a polypeptide comprising a sequence that is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical to SEQ ID NO:4 and the polypeptide has the function of TRIM and specifically binds a capsid protein of HIV.

15. A purified antibody that specifically binds to the polypeptide of any of claims 8-14.

16. The antibody of claim 15, wherein the antibody is a polyclonal antibody, a monoclonal antibody, or a chimeric antibody.

17. An antibody that specifically binds to the protein encoded by the polynucleotide of any one of claims 1-7.

18. A method of producing the polypeptide of any one of claims 8-14, which comprises:

(a) introducing a nucleic acid encoding the polypeptide into a host cell under conditions that permit expression of the polypeptide by the host cell, and

(b) recovering the polypeptide.

19. A replicable nucleic acid vector, which comprises the nucleic acid of any one of claims 1 to 7.

20. The replicable nucleic acid vector of claim 19, wherein the vector comprises a viral vector or a retroviral vector.

21. The replicable nucleic acid vector of claim 19, wherein the vector is an adenovirus vector, a retroviral vector, or an adeno-associated viral (AAV) vector.

22. A host organism comprising the replicable vector of any one of claims 19-21.

23. The host organism of claim 17, wherein the host is a prokaryote, a eukaryote, or a fungus.

24. A method for preparing a pharmaceutical composition which comprises admixing the polypeptide or a fragment thereof of any one of claims 8 to 14, thereby preparing the pharmaceutical composition.

25. A pharmaceutical composition comprising the nucleic acid of any of claims 1-7, the polypeptide of any of claims 8-14, or the vector of any of claims 19-21, and a carrier.

26. A method for treating a subject suffering from a disease or condition, which comprises administering to the subject the nucleic acid of any of claims 1 to 7, the polypeptide of any of claims 8-10, or the vector of any of claims 19-21.

27. A method for treating a subject suffering from a disease or a condition, which comprises administering to the subject a polypeptide or a fragment thereof of any one of claims 8-14, so as to treat the subject.

28. A method for preventing retroviral infection of a subject, or for treating a subject with retroviral infection, which comprises administering to the subject the pharmaceutical composition of claim 25.

29. A method for treating or preventing a viral infection of a cell, which comprises introducing a TRIM-Cyp fusion polypeptide into the cell.

30. The method of claim 29, wherein the introducing comprises tranfection of a polynucleotide encoding a TRIM-Cyp fusion polypeptide, transduction, viral-mediated introduction, or liposome-mediated introduction.

31. The method of claim 29, wherein the vial infection is an HIV infection, an HTLV infection, a pox virus infection, a retrovirus infection, a malaria infection, a hepatitis C virus infection, a hepatitis B virus infection, or a vaccinia virus infection.

32. The method of claim 31, wherein the pox virus is small pox.

33. A method for treating HIV infection or preventing HIV infection of a subject which comprises introducing into cells of the subject a TRIM-Cyp polypeptide.

34. The method of claim 33, wherein the TRIM-Cyp polypeptide binds to a capsid protein of the HIV and subsequently degrades the HIV, thereby treating or preventing HIV infection in the subject.

35. A peptidomimetic comprising an amino acid sequence substantially identical to the amino acid sequence of the polypeptide of any of claims 8 to 14 and wherein the peptidomimetic comprises TRIM function and cyclophilin function.

36. A stem cell comprising the nucleic acid of any one of claims 1 to 7, wherein the nucleic acid is part of the genome of the stem cell.

37. A method of imparting resistance to HIV to a subject or to cells of a subject which comprises administering to the cells of the subject the nucleic acid of any of claims 1 to 7, the vector of any one of claims 19-21, or the polypeptide of any of claims 8 to 14.

38. A therapeutic composition comprising the nucleic acid of any of claims 1 to 7, the polypeptide of any of claims 8 to 14, or a peptidomimetic of claim 35, and a therapeutically acceptable carrier.

39. The therapeutic composition of claim 38, wherein the carrier comprises a vector, a liposome, or a viral vector.

40. A method of *ex vivo* gene therapy which comprises: (a) removing bone marrow cells from a subject; (b) transfecting the removed bone marrow cells with a nucleic acid of any one of claims 1 to 7 *in vitro*; and (c) transplanting the transfected bone marrow back into the subject.

41. A method for reducing viral burden or load in a subject infected by a virus which comprises administering to the subject the nucleic acid of any one of claims 1 to 7, the polypeptide of any of claims 8 to 14, or the vector of any of claims 19 to 21.

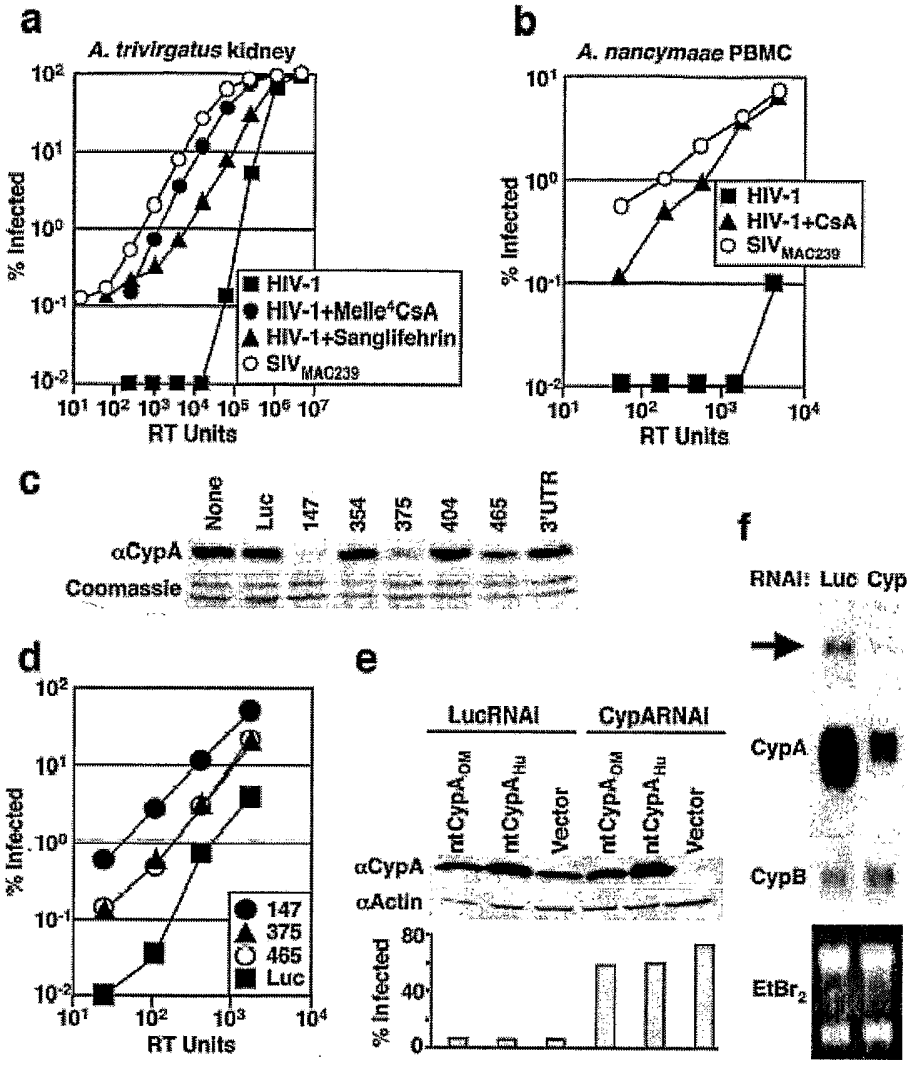
42. A topical composition for prevention or treatment of a viral infection which comprises the nucleic acid of any of claims 1 to 7 or the polypeptide of any of claims 8 to 14, and a topical carrier.

43. The topical composition of claim 42, wherein the composition is for use on the skin, in the vagina, in the nose, in the mouth, or on any skin or muscosal surface.

44. A method for reducing transposition events in a genome of a cell, which comprises introducing into the cell a polynucleotide encoding TRIM-cyclophilin A fusion protein, or

the protein encoded by the polynucleotide, so as to inhibit L1 elements and reduce transposition events in a cell.

Figure 1.


a
A. trivirgatus kidney
% Infected
RT Units
HIV-1
HIV-1+Melle4CsA
HIV-1+Sanglifehrin
SIVMAC239
b
A. nancymaae PBMC
% Infected
RT Units
HIV-1
HIV-1+CsA
SIVMAC239
c
None
Luc
147
354
375
404
465
3'UTR
αCypA
Coomassie
d
% Infected
RT Units
147
375
465
Luc
e
LucRNAi
CypARNAi
ntCypAOM
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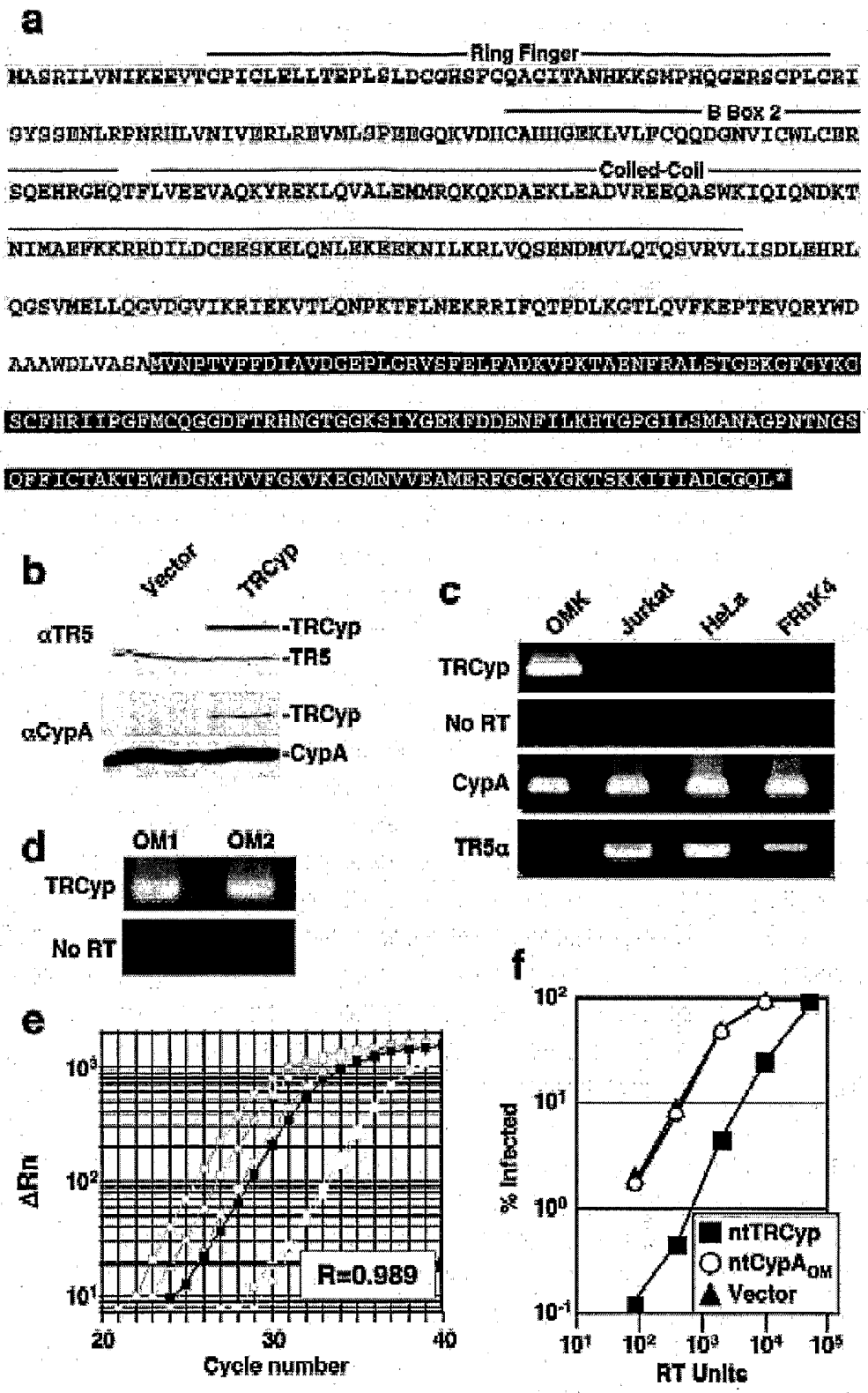
αCypA
αActin
% Infected
f
RNAi: Luc Cyp
CypA
CypB
EtBr2

Figure 2.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Ring Finger
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B Box 2
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Coiled-Coil
SQEHRGHQTFLVEEVAQKYREKLQVALEMMRQKQKDAEKLEADVREEQASWKIQIQNDKT
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SCFHRIIPGFMCQGGDFTRHNGTGGKSIYGEKFDDENFILKHTGPGILSMANAGPNTNGS
QFFICTAKTEWLDGKHVVFGKVKEGMNVVEAMERFGCRYGKTSKKITIADCGQL*
b
Vector
TRCyp
αTR5
-TRCyp
-TR5
αCypA
-TRCyp
-CypA
c
OMK
Jurkat
HeLa
FRhK4
TRCyp
No RT
CypA
TR5α
d
OM1
OM2
TRCyp
No RT
e
ΔRn
R=0.989
Cycle number
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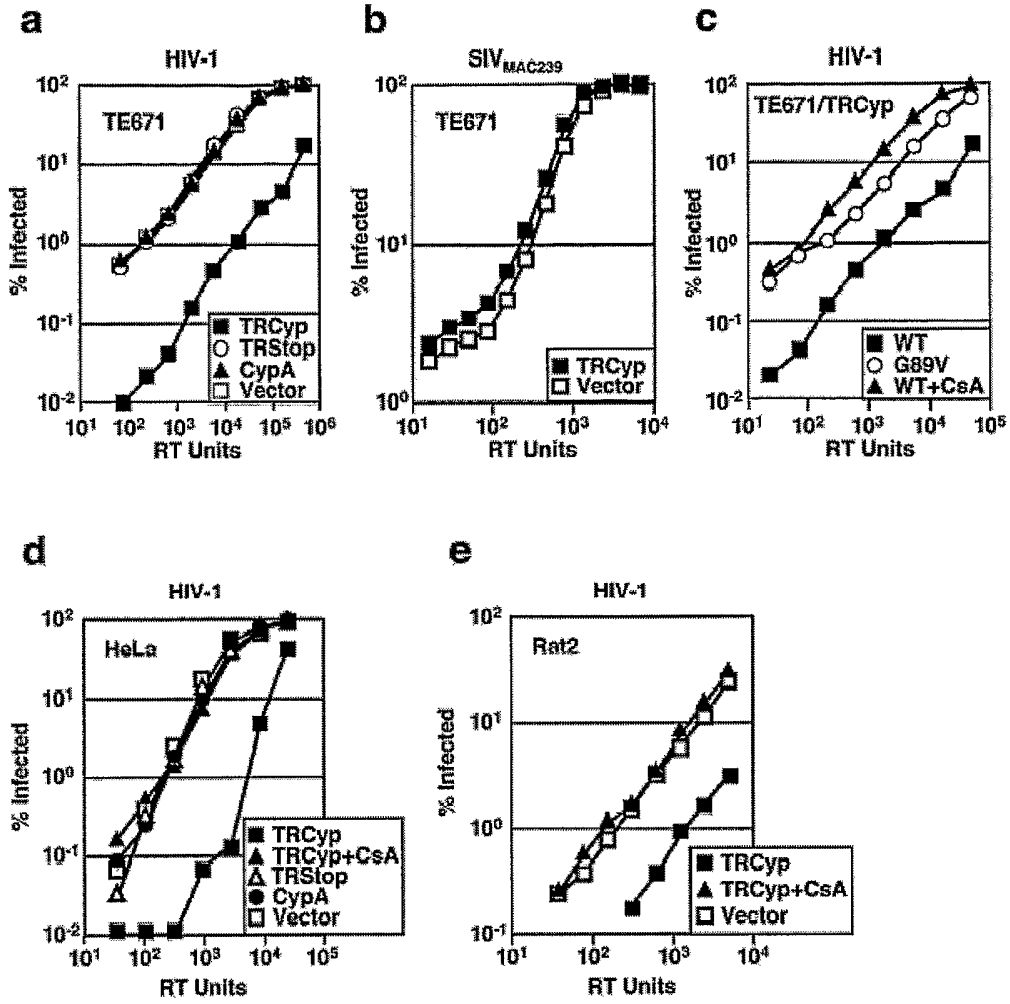
% Infected
ntTRCyp
ntCypA$_{OM}$
Vector
RT Units

Figure 3.


a
HIV-1
TE671
% Infected
RT Units
TRCyp
TRStop
CypA
Vector
b
SIV$_{MAC239}$
TE671
TRCyp
Vector
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HIV-1
TE671/TRCyp
WT
G89V
WT+CsA
d
HIV-1
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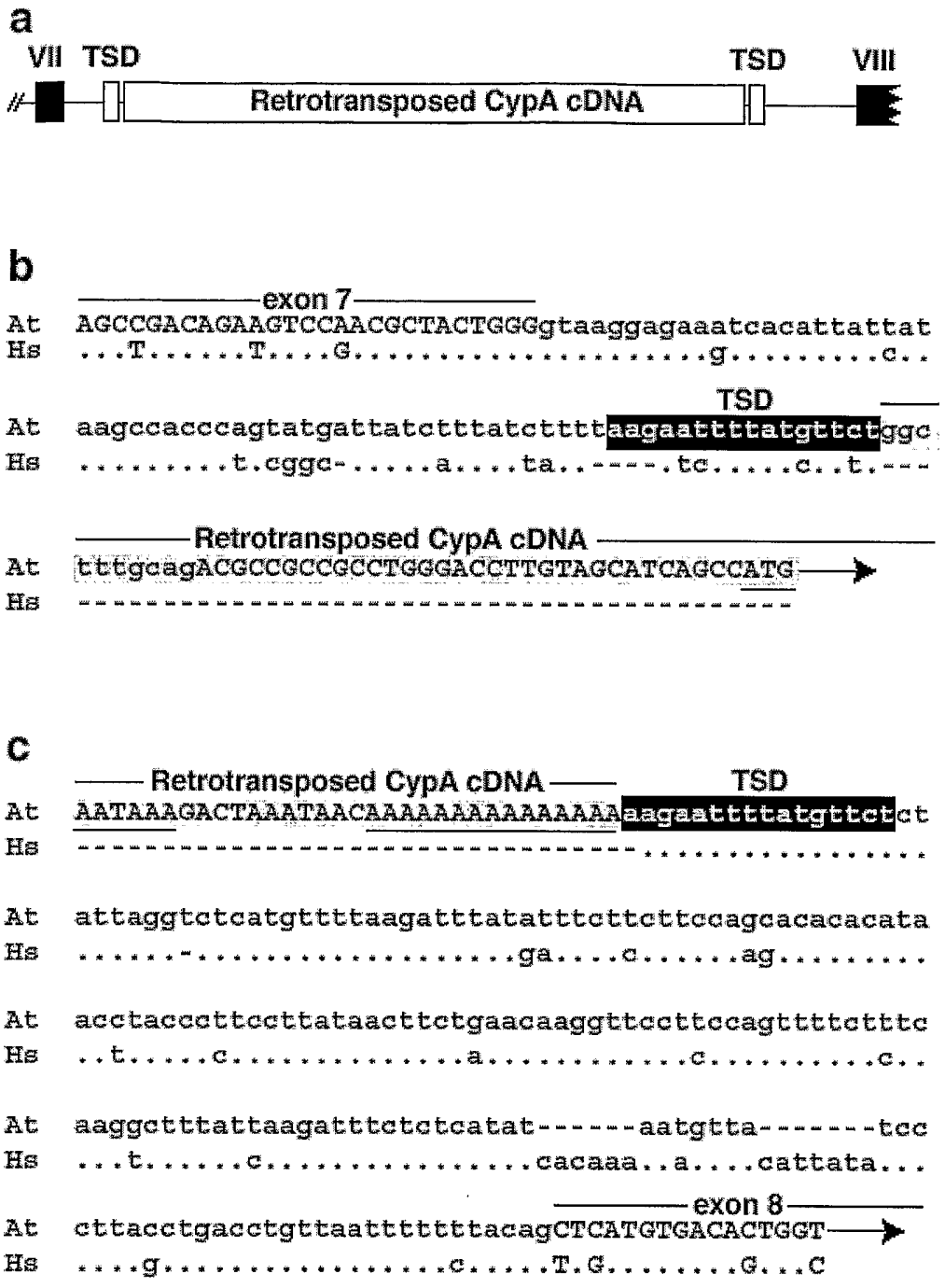
Vector

Figure 4.


a
VII
TSD
TSD
VIII
Retrotransposed CypA cDNA
b
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Hs ...T.....T....G.....g.....c..
TSD
At aagccacccagtatgattatctttatctttaagaatttttatgttctggc
Hs .....t.cggc-.....a....ta..----.tc.....c..t.---
Retrotransposed CypA cDNA
At tttgcagACGCCGCCGCCTGGGACCTTGTAGCATCAGCCATG
Hs ----------
c
Retrotransposed CypA cDNA
TSD
At AATAAAGACTAAATAACAAAAAAAAAAAAAAAaagaatttttatgttctct
Hs ----------.....
At attaggtctcatgttttaagatttatatttcttcttccagcacacacata
Hs .....-.....ga....c.....ag.....
At acctaccettcettataacttctgaacaaggttccttccagttttctttc
Hs ..t.....c.....a.....c.....c..
At aaggctttattaagatttctctcatat------aatgtta-------tcc
Hs ...t.....c.....cacaaa..a....cattata...
exon 8
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Hs ....g.....c.....T.G.....G...C

Figure 5.

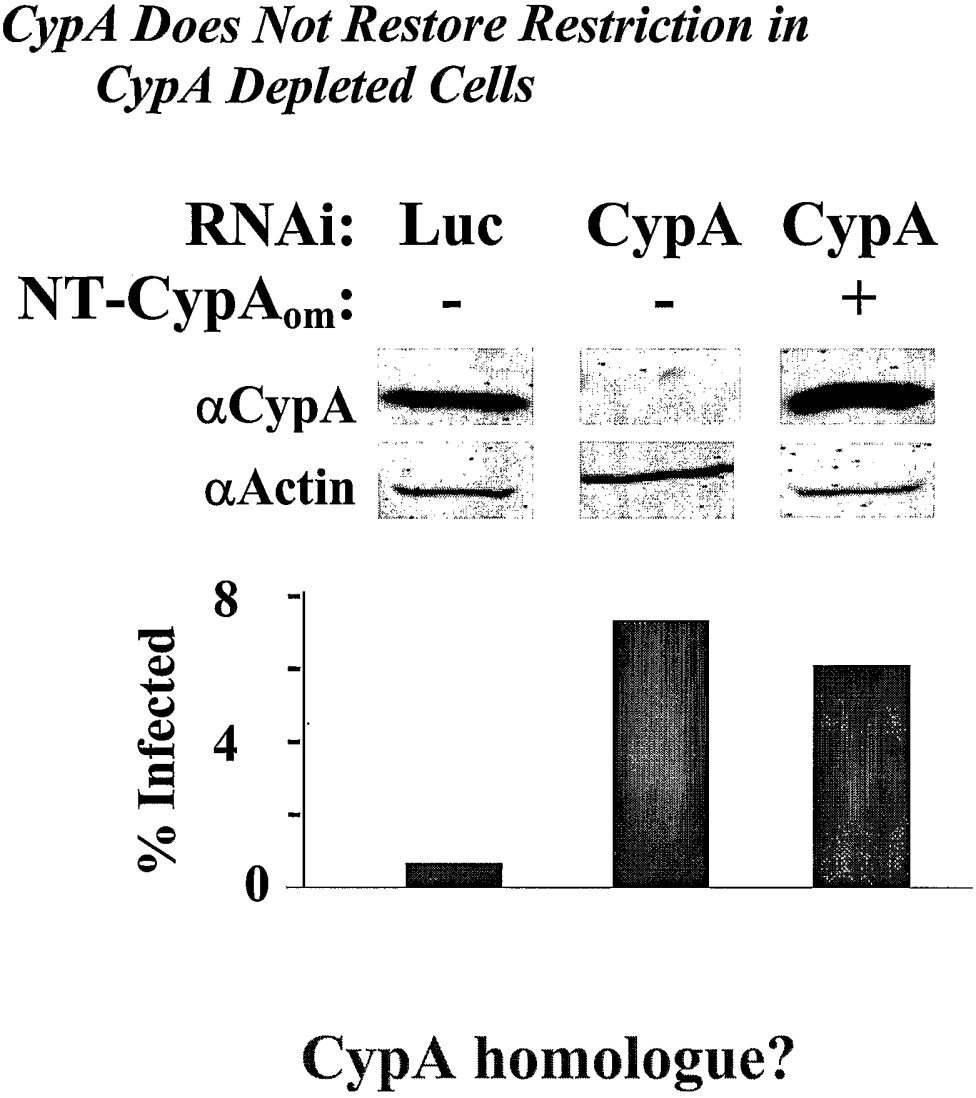

CypA Does Not Restore Restriction in CypA Depleted Cells
RNAi: Luc CypA CypA
NT-CypAom: - - +
αCypA
αActin
% Infected
8
4
0
CypA homologue?

Figure 6.

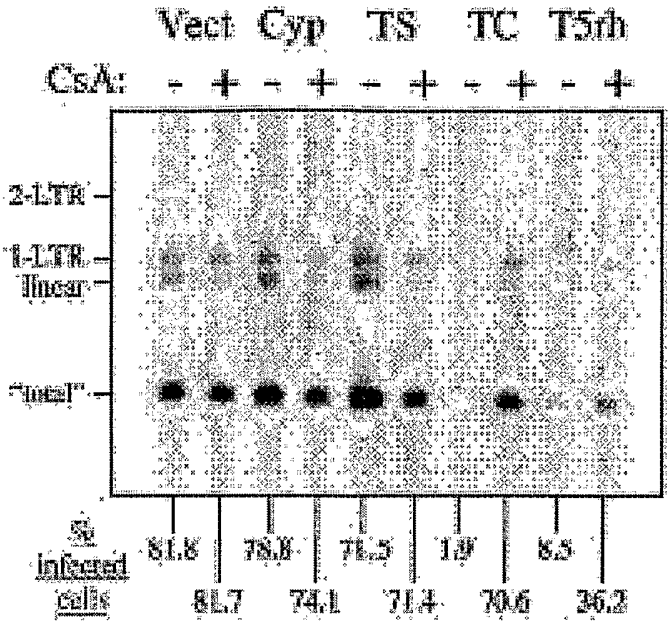

Vect
Cyp
TS
TC
T5rh
CsA:
- + - + - + - + - +
2-LTR
1-LTR
linear
"nuc"
% infected cells
81.8
78.8
71.5
1.9
8.5
81.7
74.1
71.4
70.6
36.2

Figure 7.

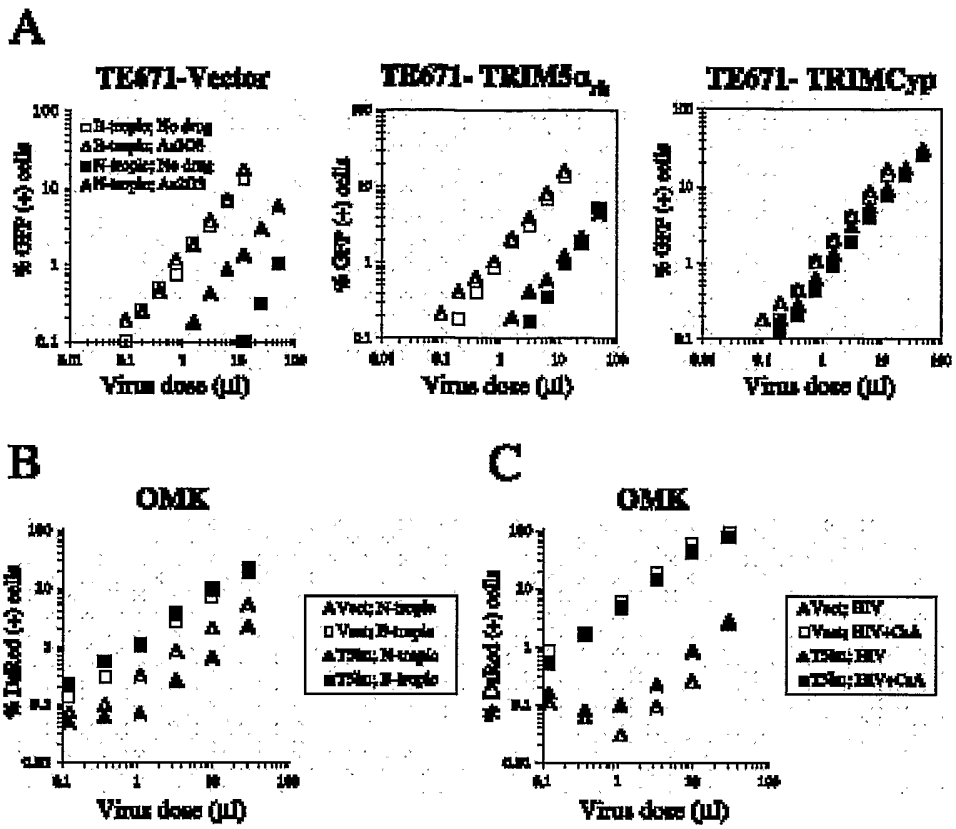

A
TE671-Vector
TE671- TRIM5α
TE671- TRIMCyp
% GFP (+) cells
Virus dose (µl)
B
OMK
% DsRed (+) cells
Virus dose (µl)
C
OMK
% DsRed (+) cells
Virus dose (µl)

Figure 8.

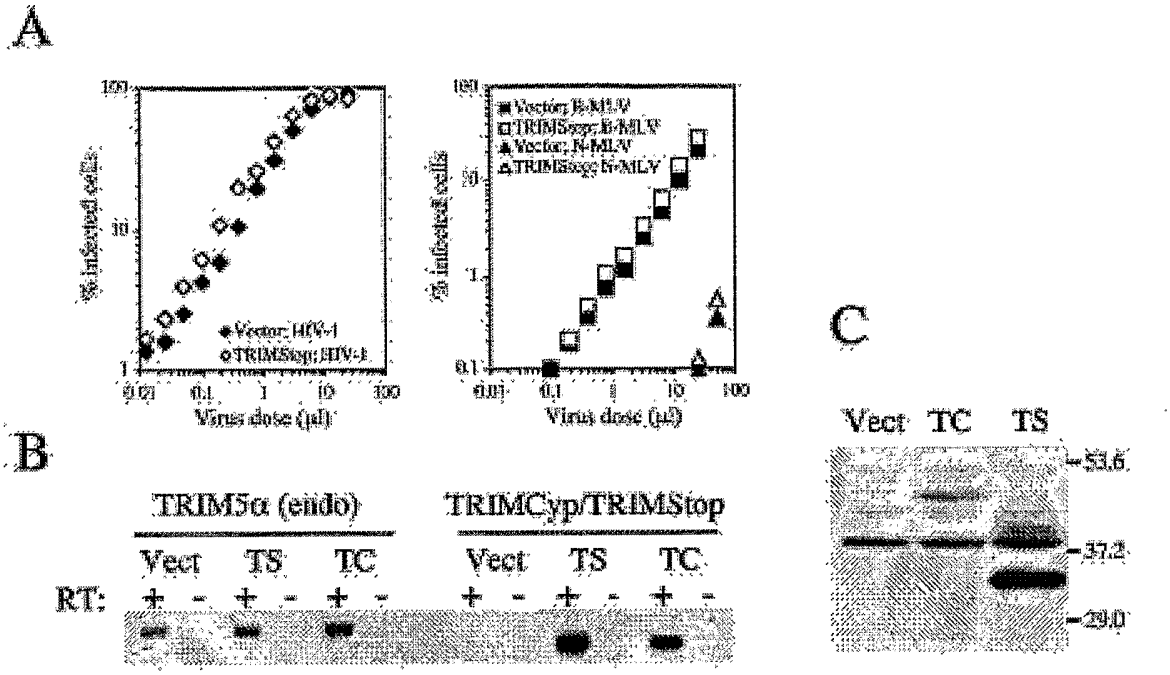

A
% infected cells
Virus dose (µl)
Vector; HIV-1
TRIMStop; HIV-1
Vector; B-MLV
TRIMStop; B-MLV
Vector; N-MLV
TRIMStop; N-MLV
B
TRIM5α (endo)
TRIMCyp/TRIMStop
Vect
TS
TC
RT:
C
Vect
TC
TS
53.6
37.2
29.0

Figure 9.

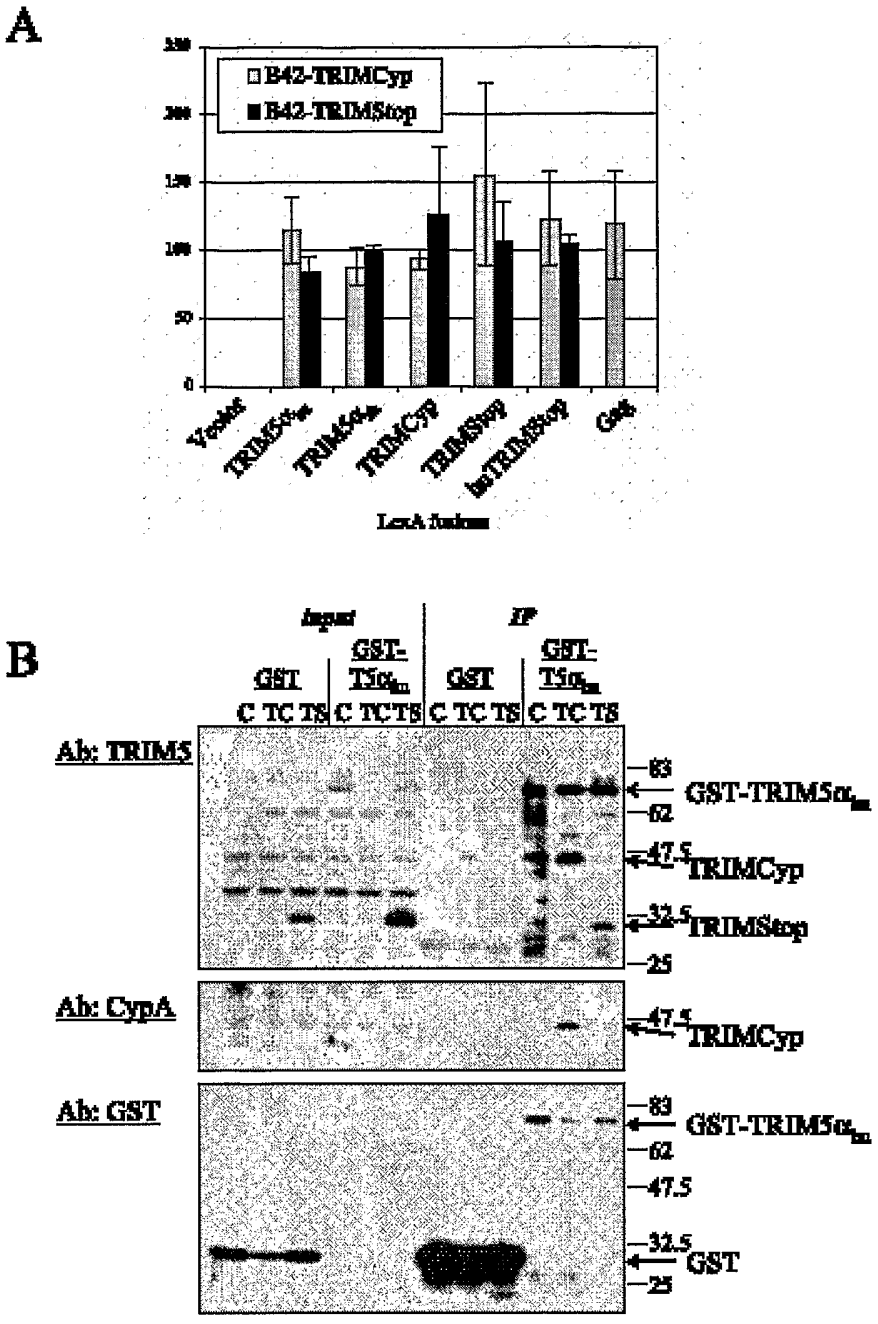

A
B42-TRIMCyp
B42-TRIMStop
Vector
TRIM5α
TRIM5α
TRIMCyp
TRIMStop
hsTRIM5cyp
Gal4
LexA fusions
B
Input
IP
GST
GST-Tsiα
C TC TS C TC TS C TC TS C TC TS
Ab: TRIM5
Ab: CypA
Ab: GST
83
62
47.5
32.5
25
GST-TRIM5α
TRIMCyp
TRIMStop
GST

Figure 10.

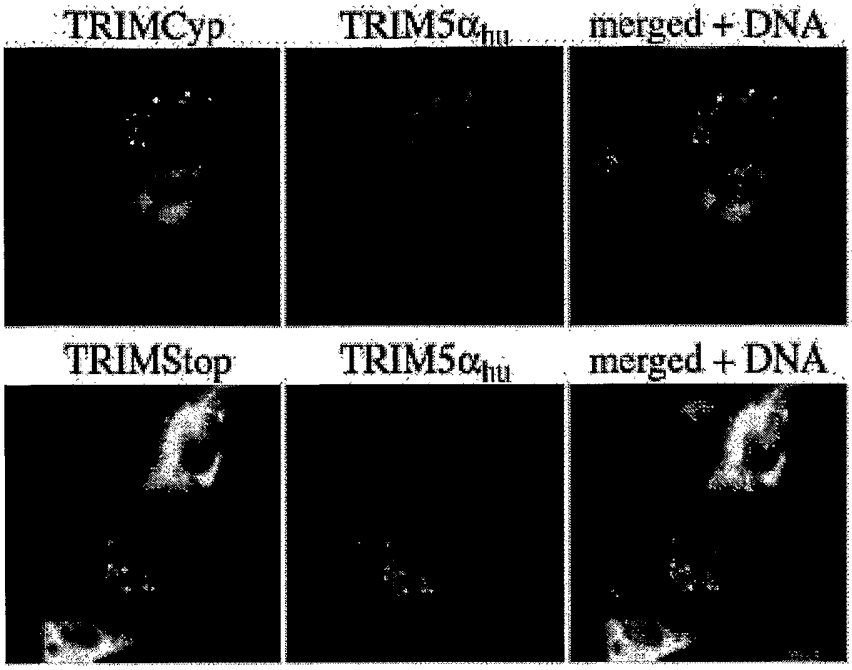


Figure 11.

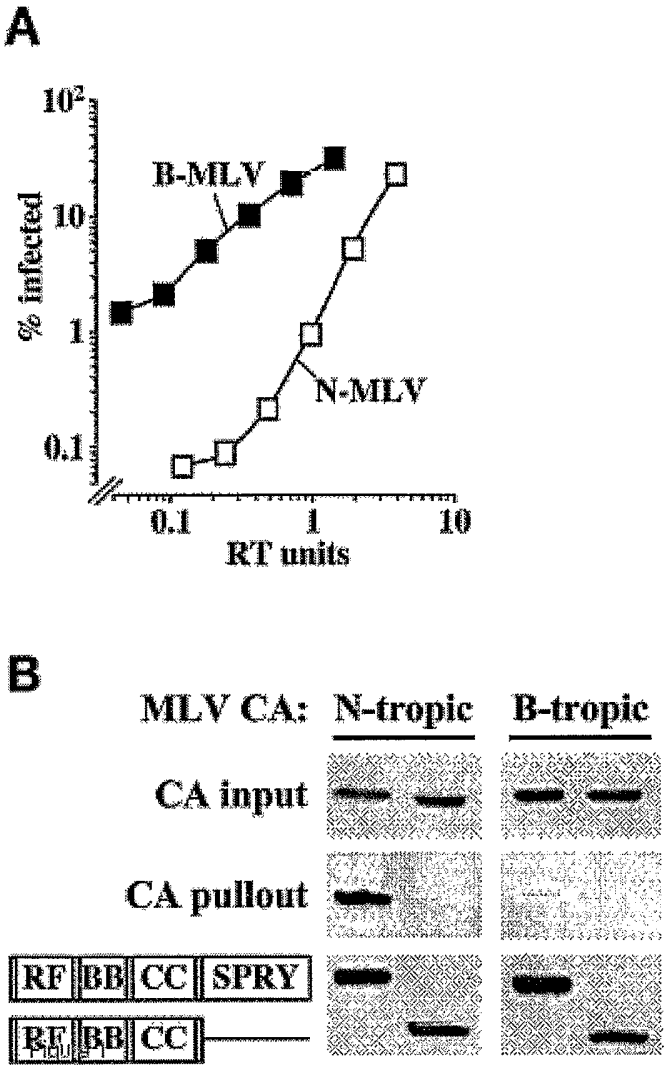

A
10^2
10
1
0.1
% infected
B-MLV
N-MLV
0.1
1
10
RT units
B
MLV CA:
N-tropic
B-tropic
CA input
CA pullout
RF
BB
CC
SPRY
RF
BB
CC

Figure 12.

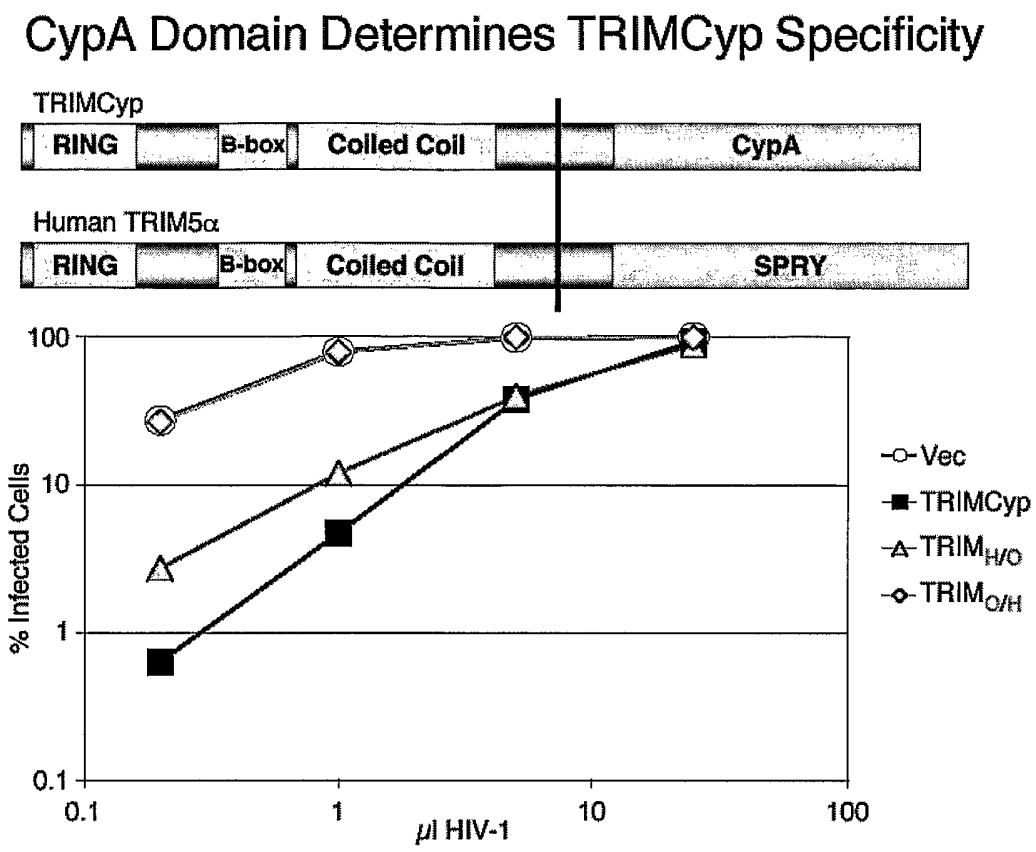
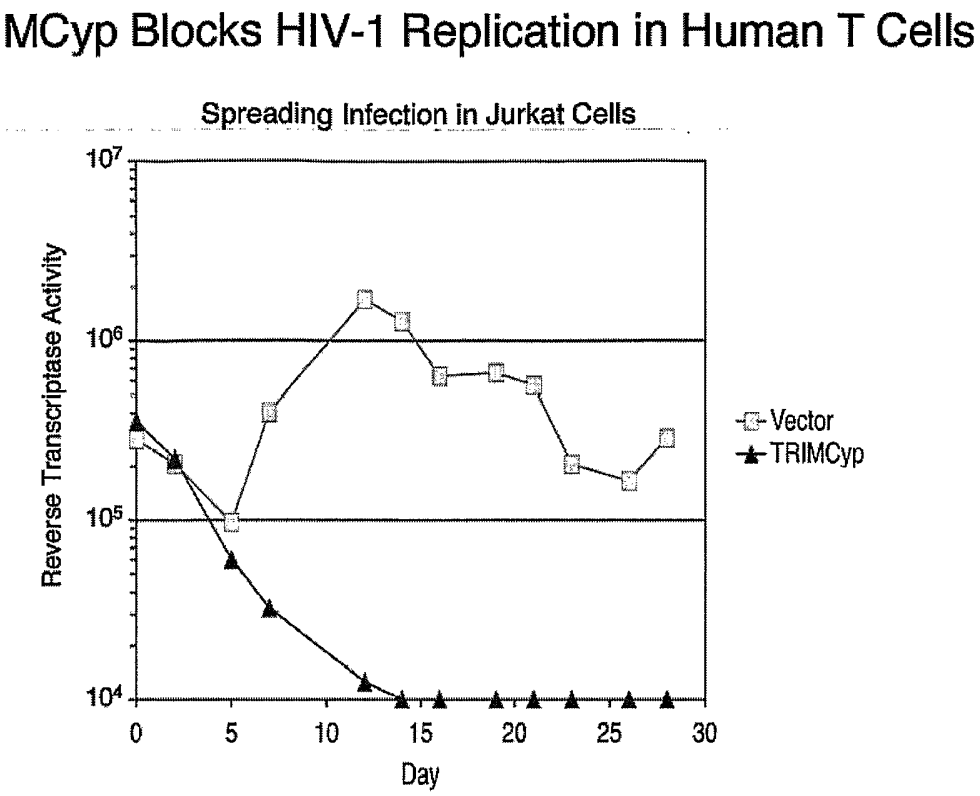

CypA Domain Determines TRIMCyp Specificity
TRIMCyp
RING
B-box
Coiled Coil
CypA
Human TRIM5α
RING
B-box
Coiled Coil
SPRY
100
10
1
0.1
% Infected Cells
0.1
1
10
100
μl HIV-1
Vec
TRIMCyp
TRIM H/O
TRIM O/H

Figure 13.


TRIMCyp Blocks HIV-1 Replication in Human T Cells
Spreading Infection in Jurkat Cells
Reverse Transcriptase Activity
10^7
10^6
10^5
10^4
0
5
10
15
20
25
30
Day
Vector
TRIMCyp