tional approaches that aim to model the dynamic nature of signaling networks, while the RNAi component pushes us closer to causal mechanistic linkages.


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**Supporting Online Material**
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Materials and Methods
SOM Text
Figs. S1 to S19
Tables S1 to S9
References


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# Restriction of an Extinct Retrovirus by the Human TRIM5α Antiviral Protein


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**Primate genomes contain a large number of endogenous retroviruses and encode evolutionarily dynamic proteins that provide intrinsic immunity to retroviral infections. We report here the resurrection of the core protein of a 4-million-year-old endogenous virus from the chimpanzee genome and show that the human variant of the intrinsic immune protein TRIM5α can actively prevent infection by this virus. However, we suggest that selective changes that have occurred in the human lineage during the acquisition of resistance to this virus, and perhaps similar viruses, may have left our species more susceptible to infection by human immunodeficiency virus type 1 (HIV-1).**


A large portion of primate genomes is composed of endogenous retroviruses that can be thought of as an archaeological record of past infections. Both chimpanzee and gorilla genomes harbor more than 100 copies of *Pan troglodytes* endogenous retrovirus (PtERV1), whereas it is absent from the human genome (*1*). Comparison of individual PtERV1 proviruses in gorilla and chimpanzee genomes suggest that this virus was active 3 to 4 million years ago, after the separation of chimpanzee and human lineages (*1*). This raises an evolutionary conundrum as to why sister species, but not humans, acquired germline copies of this retrovirus even though all three species cohabited when PtERV1 was an active exogenous virus (*1*). One mechanism of active restriction from retroviral infections is conferred by the TRIM5α protein, which binds directly to the incoming retroviral capsid (CA) core and targets its premature disassembly or destruction (*2*, *3*). Each primate species encodes a TRIM5α with a different antiviral specificity (*4*). For example, TRIM5α encoded by rhesus macaques renders them resistant to infection by HIV-1, but human TRIM5α affords no such protection (*5*). Indeed, the antiviral specificity of TRIM5α has rapidly evolved by dramatic episodes of positive selection during the past 30 million years of primate evolution (*6*). The branch leading to the human lineage shows one of the strongest signatures of positive selection (*6*), which suggests that at least one major pathogenic retroviral assailant has challenged the human lineage in the past 4 to 5 million years. Taken together, these findings suggest that TRIM5α evolution was shaped by a species-specific history of ancestral retroviral challenges. Although human TRIM5α has relatively poor activity against retroviruses compared with the gene from other primates, it potently blocks a γ-retrovirus N-MLV, which is related to PtERV1 (*7*, *8*). We therefore tested the hypothesis that TRIM5α may have protected early humans from invasion by PtERV1.

All copies of PtERV1 in the chimpanzee genome have been inactivated by accumulated detrimental mutations (*1*). However, the numerous proviral copies of PtERV1 present in the chimpanzee genome allow us to reconstruct the ancestral sequence of the *gag* gene of this ancient, extinct retrovirus in silico (see supporting online material). Analysis of the reconstructed PtERV1 ancestral sequence reveals about 50% identity with murine leukemia virus (MLV), and several characteristic conserved elements are intact (Fig. 1A). Phylogenetic analysis of chimpanzee and gorilla PtERV sequences shows that a single-source virus likely infected both chimpanzees and gorillas because viral sequences from both species form a monophyletic group (Fig. 1B).

We next used site-directed mutagenesis to reconstruct the ancestral p12 and CA coding regions (ignoring synonymous changes) starting from one chimpanzee PtERV1 provirus cloned from the genome. We focused on CA because it is the functional target of TRIM5α and included p12 because of functional interactions that exist between p12 and CA in other γ-retroviruses (*9*). Because TRIM5α interacts with the retroviral CA only in the multimeric structure characteristic of mature retroviral particles (*10*), we generated the PtERV1 capsid core in the context of an infectious virus capable of only a single round of infection. This was achieved by constructing a chimeric virus between PtERV1 and MLV that encodes a *gag/pol* gene expressing the reconstructed p12 and CA proteins of PtERV1 with the remainder of the viral structural proteins and enzymes of MLV (Fig. 1C). Our MLV/PtERV1 chimeric virus was indeed infectious (Fig. 1D), which demonstrates that regions of a 3- to 4-million-year-old primate endogenous retrovirus can be successfully resurrected.

We tested human TRIM5α restriction of PtERV1 by infecting cells that express an exogenous copy of human TRIM5α. A much younger human endogenous retrovirus, HERV-K, was also recently resurrected (*11*, *12*) but was not restricted by human TRIM5α (*12*). In contrast, expression of human TRIM5α in a heterologous cell type resulted in a dramatic reduction of infectivity of the MLV/PtERV1 chimera by a factor of more than 100 compared with cells that do not express TRIM5α (Fig. 2A). These data indicate that humans possess an intrinsic immunity gene capable of effectively neutralizing an extinct retrovirus that never successfully fixed into the human genome.

Specificity of TRIM5α for a particular retroviral capsid is largely determined by amino acids within the C-terminal B30.2 domain. Within this


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capsid-interaction domain, a particular "patch" contains amino acids under positive selection that contain the dominant determinants of specificity (*6*, *13*, *14*). For example, the amino acid at position 332 within this patch is a critical determinant of HIV-1 restriction (*13*). Humans and chimpanzees encode an arginine (R) residue at position 332, whereas the hominoid ancestral residue at this position is a glutamine (Q). Reversing this

**Fig. 1.** Reconstruction of PtERV1 p12 and CA and infectivity of PtERV1/MLV chimeric virus. (**A**) An alignment of the p12 and CA sequences of the consensus PtERV1 from chimpanzee (top line) and MLV (bottom line) is shown. A late domain (PPPY) characteristic for γ retroviruses is in p12 and the Major Homology Region (MHR) conserved in all retroviruses is present in CA are indicated by shaded boxes. (**B**) An unrooted phylogenetic NJ tree was generated with nucleotide sequences of PtERV1 (MA through PRO) from chimpanzee, rhesus, and gorilla genomes. Branches are color coded: gorilla, orange; chimpanzee, blue; and rhesus, green. Fifteen amino acid changes were introduced into one extant copy of PtERV1 p12-CA from chimpanzees to generate the "ancestral" clone (fig. S1). The derived consensus sequence from chimpanzee is denoted by a black branch. The nucleotide consensus is slightly displaced from the node of the monophyletic clade containing chimp and gorilla sequences because synonymous changes were not introduced during the reconstruction process. (**C**) Schematic representation of the chimeric virus between MLV and PtERV1 is shown. The Gag-Pol expression vector used to make virus contains the MA, NC, and the entire POL coding sequence of MLV and the p12 and CA from PtERV1. Protease cleavage sites between MLV MA and PtERV1 p12, as well as between PtERV1 CA and MLV NC, were generated to match cleavage sites recognized by MLV protease. (**D**) Green fluorescent protein (GFP)–encoding virus was used to infect Crandell-Rees feline kidney (CRFK) cells with PtERV1 chimeric virions. Fluorescence-activated cell sorting (FACS) plots of virus-challenged cells are shown. The *y* axis indicates cell size, and the *x* axis denotes GFP positivity, which is an indicator of transduction. The initial PtERV1 clone used to reconstruct this consensus was tested in the chimeric context and was noninfectious (left). After 15 mutations were introduced to reconstruct the consensus p12 and CA sequence, infectious virus particles were generated (right).

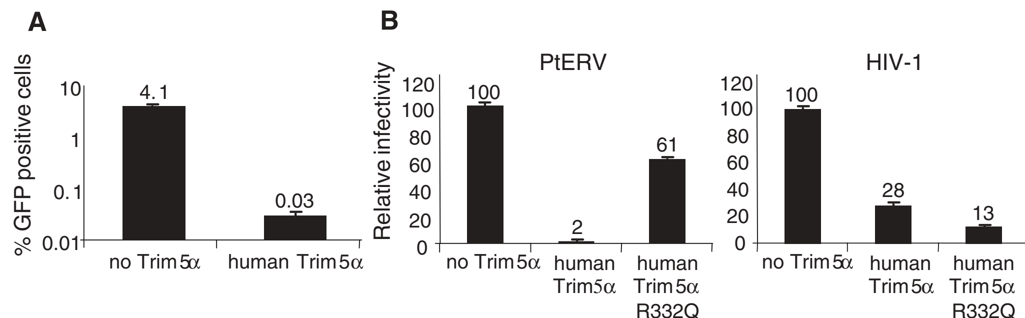


**Fig. 2.** Human TRIM5α restricts PtERV1 and restriction maps to a single residue within the interaction domain. (**A**) TRIM5α cloned from human cDNA was stably transduced into feline CRFK cells. These human TRIM5α-expressing cells along with CRFK control cells were infected with a GFP-encoding PtERV1 in a single-round infection assay. (**B**) Human TRIM5α with an R332Q mutation was generated and stably expressed in CRFK cells. These cells, along with cells expressing wild-type human TRIM5α, were used as targets for GFP-encoding PtERV1 or HIV-1. Infections were done in triplicate.

change (R332Q) had moderate effects on the ability of human TRIM5α to restrict MLV variants (fig. S2). Notably, changing the arginine to the ancestral glutamine abolished the ability of human TRIM5α to efficiently restrict PtERV1 infectivity (Fig. 2B). Unexpectedly, the R332Q mutation had the opposite effect on HIV-1, improving the ability of human TRIM5α to restrict this virus (*15*) (Fig. 2B). Thus, the R332Q mutation in human TRIM5α reveals a trade-off in TRIM5α's ability to restrict two retroviruses; a mutation that abolished restriction for PtERV1 results in a gain of restriction to other viruses such as HIV-1.

We tested the idea that restriction of PtERV1 and HIV-1 by TRIM5α is mutually exclusive by cloning and testing a panel of TRIM5α genes from other primates (Fig. 3, A and B). Indeed, we found no case where a primate encodes a TRIM5α capable of efficient restriction of both PtERV1 and HIV-1 (Fig. 3A). This is especially notable in TRIM5α from Old World monkeys where, for example, TRIM5α from baboons and African green monkeys have fifty-fold restriction of HIV-1 but negligible restriction of PtERV1. In contrast, that from sooty mangabeys has a fifty-fold restriction of PtERV1 but negligible restriction of HIV-1 (Fig. 3A). The same effect, although less extreme, was seen in the hominoid TRIM5α alleles, except in orangutan and gibbon, which are poor restrictors of both viruses. Thus, during primate evolution, a gain of restriction for one virus appears to have coincided with a loss of restriction against another virus.

Although we cannot rule out the possibility that PtERV1 never infected human ancestors for other reasons (SOM Text, note 1), our data do suggest the possibility that TRIM5α was fixed in human populations because of its ability to confer protection against PtERV1 (Figs. 2 and 3) and that modern humans have descended from ancestors who resisted infection. Indeed, we know that there is very little diversity in the human population today in the part of TRIM5α that determines antiviral specificity (*6*, *16*, *17*). However, we find that chimpanzee TRIM5α is also capable of restricting PtERV1 and encodes an R332 (Fig. 3), yet chimpanzees contain multiple copies of PtERV1 in their genome and humans do not. Moreover, we find that R332 is monomorphic in the TRIM5α allele in all four subspecies of chimpanzees and in bonobos, which indicates that R332 is evolutionarily conserved through the chimpanzee radiation (in the past 1 to 2 million years). The most parsimonious explanation for the presence of R332 in humans and chimpanzees is that the mutation was fixed in our common ancestor, which presents a paradox because chimpanzee TRIM5α did not protect them against PtERV1. This suggests that TRIM5α alone does not determine retroviral invasion into the germline but that the combination of multiple retroviral restriction factors that are also rapidly evolving, such as the Apobec3 family (*18*), are necessary to explain ancient transmission events.

A second evolutionary scenario is that the human and chimp lineages independently fixed R332 after the species diverged. We know that the amino acid at position 332 has indeed changed independently in many different primate lineages, even among very closely related species (*6*, *19*). Moreover, at least two Old World monkey species harbor multiple alleles of TRIM5α that are polymorphic at position 332 and are likely maintained by balancing selection (*20*). Given the intensity of positive selection acting on this position, the less parsimonious explanation of independent fixation is not unlikely. In this scenario, infections in the chimpanzee lineage subsequent to PtERV1 could have driven the selection of R332.

Finally, our data show that resistance to PtERV1 comes at a cost because it reduces resistance to other retroviruses, such as HIV-1. Although retroviruses similar to HIV-1 are also found in chimpanzees and in gorillas (*21*, *22*), we do not yet know how the degree of antiviral activity conferred by TRIM5α relates to resistance or sensitivity to infection at the organismal level. Nonetheless, our analyses demonstrate that selection for resistance to one potential retroviral pathogen could render a host defense gene less capable of inhibiting another virus. We speculate that fixation of a mutation in human TRIM5α, which could have protected early humans from viruses such as PtERV1 in our distant evolutionary past, may be at least partially responsible for our currently poor resistance to HIV-1.

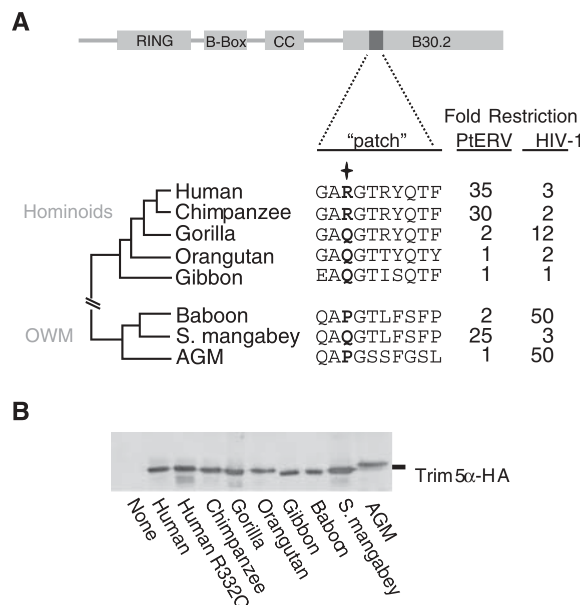


**Fig. 3.** Mutually exclusive restriction of PtERV1 and HIV-1 by primate TRIM5αs. (**A**) Primate TRIM5α genes were cloned, and hemagglutinin (HA)–tagged cDNAs were stably expressed in CRFK cells. Each stable cell line was assayed for restriction by PtERV1 or HIV-1 in a single-round infection assay using GFP-encoding viruses. Shown is a schematic representation of TRIM5α protein domains. The sequence of amino acids ("patch") within the B30.2 interaction domain that partially determines retroviral specificity is indicated for each primate tested, along with a cladogram of accepted primate phylogeny including the hominoid clade (top) and selected Old World monkeys (bottom). The asterisk indicates amino acid 332. Restriction of PtERV1 or HIV-1 is expressed as average fold restriction of three independent infections compared with a no-TRIM5α control. SDs were negligible. (**B**) Expression of each primate TRIM5α was similar, as detected by Western Blot analysis for the N-terminal HA-epitope tag.

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Materials and Methods
SOM Text
Figs. S1 and S2
References

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