AIDS Reviews 2007;9:114-25

A Brief History of TRIM5α

Ruchi M. Newman and Welkin E. Johnson

Division of Microbiology, New England Primate Research Center, Department of Microbiology and Molecular Genetics, Harvard Medical School, Southborough MA, USA

Abstract

In spite of the fact that the first isolates of HIV-1 became available more than 20 years ago, there is still no robust animal model for HIV-1 replication and pathogenesis. This is largely due to the existence of multiple genetic barriers to HIV-1 replication in most nonhuman primates, including a severe block targeting the early, post-entry phase of the viral replication cycle. It is now known that a protein called TRIM5α mediates this early restriction in nonhuman primate cells. Tissue culture experiments, together with genetic association studies involving multiple HIV/AIDS cohorts, indicate that the human orthologue of TRIM5α does not have a significant impact on HIV-1 replication. However, most human alleles encode a functional protein that can restrict at least one retrovirus unrelated to HIV-1 (N-tropic murine leukemia virus), although one deleterious mutation (H43Y) is present at high frequency in human populations. Phylogenetic analyses of the TRIM5 locus reveal that prehistoric retroviral epidemics, not unlike the current HIV/AIDS pandemic, played a significant role in the evolutionary history of humans and their primate relatives. The discovery of TRIM5α's antiretroviral activity sparked the imaginations of many laboratories, and considerable effort has now been channeled into characterizing the protein and determining its possible mechanism(s) of action. It is hoped that research on TRIM5α will contribute to the establishment of new and improved models for HIV replication and AIDS pathogenesis, point the way towards novel therapeutic targets to stem the tide of the human AIDS epidemic, provide an experimental window onto the early, post-entry stages of the retroviral replication cycle, and even inspire the search for other cellular factors that modulate retroviral infection. **(AIDS Reviews 2007;9:114-25)**

Corresponding author: Welkin E. Johnson, wjohnson@hms.harvard.edu

Key words

TRIM. TRIM5α. Tripartite motif. Retrovirus. Restriction factor.

Introduction

Soon after isolates of HIV-1 became available to the biomedical community in the mid-to-late 1980s, researchers became aware of a genetic barrier to HIV-1 infection of cells derived from Old World monkeys. Although several laboratories would go on to characterize the block in great detail (including, no doubt, numerous unheralded attempts to clone the responsible gene), it was not until 2004 that the primate *TRIM5* gene was identified as the culprit. Within 36 months of the initial publication describing TRIM5α-mediated restriction of HIV-1[96], more than 70 articles related to TRIM5α and retroviral restriction appeared in the primary, peer-reviewed literature. A retrospective look at the search for this gene, and the flurry of publications that appeared in the wake of its discovery, reveals that time and time again key insights were driven by the extraordinary evolutionary history of the *TRIM5* locus. Our current understanding of TRIM5α-mediated restriction of HIV-1 and other retroviruses comes from a remarkable synthesis of experimental approaches, including comparative virology, molecu-

Correspondence to:
Welkin E. Johnson
Division of Microbiology
New England Primate Research Center
Department of Microbiology and Molecular Genetics
Harvard Medical School
One Pine Hill Drive
Southborough, MA 01772, USA
E-mail: wjohnson@hms.harvard.edu

lar biology, biochemistry, population-genetics, and phylogenetic analysis.

The "Monkey Block"

While attempting to identify potential animal models for HIV-1 infection and pathogenesis, HIV/AIDS researchers discovered that the host-range of HIV-1 was effectively limited to humans and apes[3,24,25,27,59]. In contrast, a related lentivirus (SIVmac) that had been recently isolated from captive rhesus macaques, could replicate in both human and monkey cells[17,26]. By the early 1990s, it was found that recombinant viruses, derived by combining portions of the SIVmac and HIV-1 genomes, would replicate in monkey cells so long as the region encompassing the viral *gag, pol* and *vif* genes was derived from the SIV parent[38,51,84-86]. Thus, the viral determinant(s) of restriction in monkey cells were known to map to some or all of these genes, and the block(s) were not related to the initial entry event mediated by the viral envelope protein.

Hoffman, et al.[39] surveyed a large panel of mammalian cell lines for susceptibility to infection with single-cycle versions of HIV-1 and SIVmac; these included cells derived from humans, a variety of other Old World primates (including apes and monkeys of both African and Asian origin), and New World primates (Central and South American monkeys). The viruses were pseudotyped with a promiscuous envelope protein from an unrelated virus (VSV-G), thus bypassing blocks related to cell-surface binding, fusion and entry. This particular screen, therefore, specifically revealed the presence of barriers to the first half of the retroviral replication cycle, a stage encompassing reverse-transcription, integration and expression. They found that single-cycle HIV-1 infection was blocked in most Old World monkey (OWM)-derived cell lines, while SIVmac infection was blocked in cell lines originating from New World monkeys (NWM). Cells from owl monkeys *(Aotus sp.)* presented a notable exception to this pattern: unlike cells from other New World species, single-cycle HIV-1 infection was blocked in owl monkey cell lines.

Subsequent studies demonstrated that the blocks in most cases were due to the presence of dominant, inhibitory factors, and that restriction could be transiently overcome by pre-incubation of target cells with an excess of restriction-sensitive virions[5,15,63]. Because the block was initially defined as a genetic barrier to lentiviral infection in primate cells, it was named lentivirus susceptibility factor-1 (Lv-1)[15].

A similar experimental strategy led to the detection of a block to infection of N-tropic murine leukemia virus (N-MLV) in human cell lines, and this restriction was dubbed Ref-1 (restriction factor-1)[101]. Characterization of Lv-1 and Ref-1 revealed similarities in the nature and timing of the block imposed by the two loci, and were consistent with the possibility that Ref-1 and Lv-1 might be human and monkey versions of the same gene[8].

A key piece of the puzzle fell into place with the discovery that a protein called TRIM5α was somehow responsible for the block. TRIM5α was identified during a screen for cDNA clones that would protect human cells from HIV-1 infection[96]. For this screen, a cDNA library was derived from HIV-resistant, rhesus macaque lung fibroblasts and introduced into an HIV-sensitive, human cell line (HeLa). The transduced cells were then challenged with VSV-pseudotyped, single-cycle HIV-1, and resistant cells were pooled and subjected to further rounds of selection. Resistant clones that emerged from this screen were found to harbor cDNA encoding the rhesus orthologue of TRIM5α. Introduction of rhesus TRIM5α into permissive cell lines resulted in restriction of HIV-1 but not SIVmac or an SIV/HIV chimera containing the capsid domain of SIV. Additionally, siRNA-mediated knockdown of endogenous TRIM5α expression in rhesus cells relieved the early, post-entry block to HIV-1 infection. In short order, work done in a number of laboratories quickly confirmed that Ref-1 and Lv-1 were indeed the human and monkey orthologues of TRIM5α[36,47,76,93,109].

Around the same time, Sayah, et al.[82] succeeded in tracking down the cause of the unusual block to HIV-1 infection in owl monkey cells. They also traced restriction to the *TRIM5* locus; however, the owl monkey version of the gene encoded a TRIM5-cyclophilin A fusion protein (TRIMCyp). This same group, and others, had already documented that HIV-1 capsid (CA) binds to cellular cyclophilin A (CypA), and that CypA normally acts as a positive cofactor for HIV-1 replication in human cells[10-13,19,20,22,23,58,99]. This interaction is disrupted by CypA-binding drugs such as cyclosporin A (CsA) and, as it turns out, restriction by the TRIMCyp fusion protein is also inhibited by treatment with CsA. Thus, it seemed likely that the CypA domain served to target TRIMCyp to incoming virion capsids, and supported the notion that the C-terminal B30.2/SPRY domain of TRIM5α served a similar targeting function. For a detailed review of the discovery of TRIMCyp and the complex relationship between HIV-1 replication, CypA binding and TRIM5α-mediated restriction, the reader is directed to recent articles by Sokolskaja and Luban[90] and Luban[57].

Table 1.

Source of TRIM5α		HIV-1	N-MLV	SIVmac	References
Hominids	Human	+	+++	+	36,65,72,81,91, 93,96,109
	Chimpanzee	+	+++	–	93, 68
	Gorilla*	++	+++	+++	68
	Orangutan	+	+++	?	93, 68
Old World monkeys	Rhesus macaque	+++	+++	+	36,65,72,81,91, 93,96,109
	Pigtailed macaque*	+++	+++	–	68
	AGM(pyg)*	+++	+++	+	93
	AGM(tan)	+++	+++	+++	68,91,93
	AGM(CV-1 line)*	+++	+++	+++	36
	Sooty mangabey	+++	+++	–	65,68
New World monkeys	Spider monkey*	+++	+++	+	93
	Tamarin (red-chested)*	+	+	+++	93
	Tamarin (cotton top)*	+++	+++	+++	68
	Tamarin (emperor)*	+++	+++	+++	68
	Squirrel monkey	–	–	+++	93,91

–: no restriction; +: weak restriction; ++: moderate restriction;+++: strong restriction; AGM -African green monkey; ?: not reported.
*result is based on one study.

The TRIM family of proteins

TRIM5α is one member of a large family of related proteins involved in various cellular functions. All members of the TRIM family of proteins contain three discrete domains, including an N-terminal RING domain followed by one or two B-boxes and a coiled-coil region, which are collectively called the RBCC motif. Large differences between TRIM family members occur mainly at the C-terminus, which can be comprised of a variety of unrelated protein domains thought to be involved in protein-protein interactions[67,77].

While over 68 different TRIM family members have been identified in primates, mice and other species, very little is known about their physiological roles. Many RBCC family proteins have been shown to form higher order complexes that define specific cellular compartments[77]. Dysregulation and mutations of other TRIM family members have been linked to a variety of pathological conditions, including genetic diseases and oncogenesis. Additionally, some primate TRIM proteins (TRIM1, TRIM5α, TRIMCyp, TRIM19, TRIM22, TRIM32, TRIM34) reportedly have antiviral activity, and may act to inhibit different viruses by a variety of proposed mechanisms[67]. Of these, TRIM5α has generated great interest among HIV/AIDS researchers since it is known to impose a major block to lentiviral infection of Old World monkeys (Table 1)[36,47,96,109].

TRIM5α

TRIM5α is the longest of four splice variants of the *TRIM5* gene and falls into the subset of TRIM family proteins with a C-terminal B30.2/SPRY domain. While TRIM5 is ubiquitously and constitutively expressed, no additional cellular function has been attributed to this protein[77]. However, its similarity to a large family of related TRIM proteins implicated in diverse cellular processes leaves open the possibility that TRIM5 may participate in other, as yet unidentified, functions.

Upon infection with a susceptible retrovirus, TRIM5α is thought to hinder viral infection post-entry but prior to integration of viral DNA into the host genome[7,31,67] (Fig. 1). Recent studies using proteasome inhibitors suggest that TRIM5α may play an additional role in targeting the pre-integration complex for degradation by the proteasome

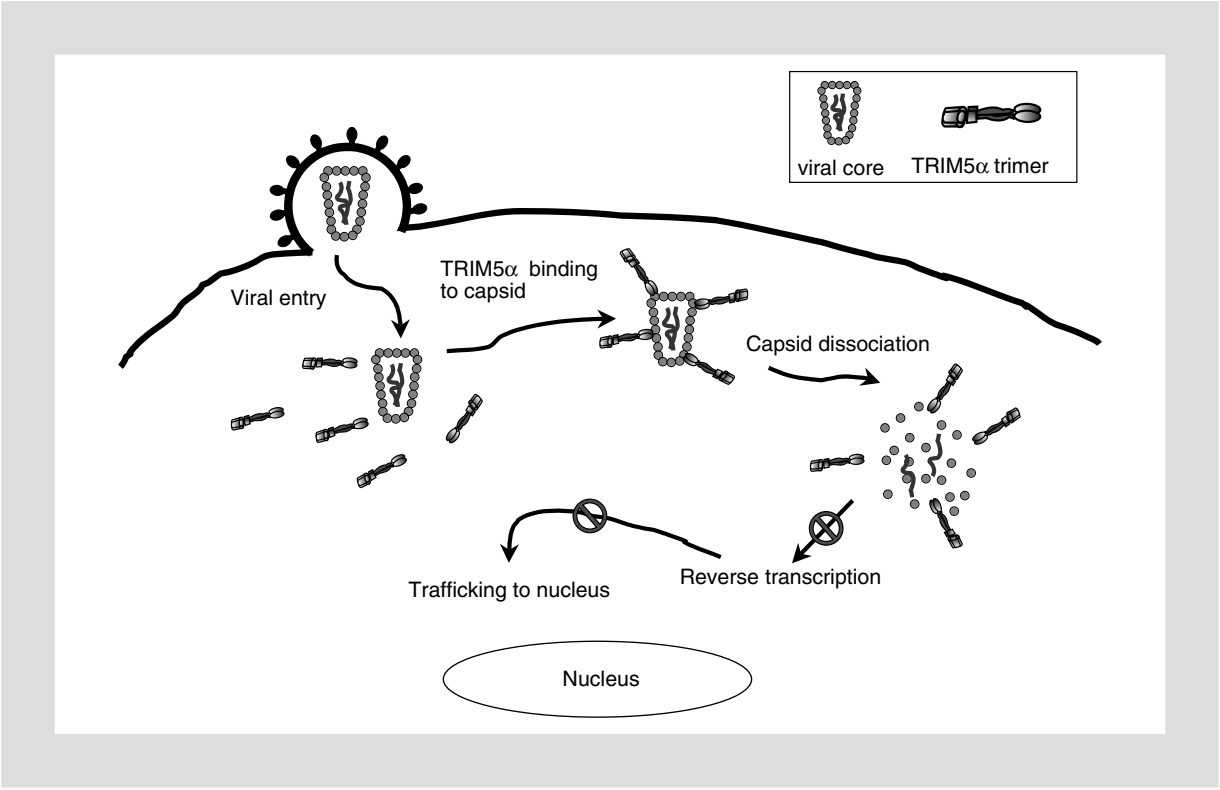

Figure 1. *Post-entry restriction of retroviral infection by TRIM5α. After being released into the cytoplasm of a target cell, retroviral cores are recognized by trimers of TRIM5α. TRIM5α binding to capsid is thought to mediate premature disassembly of capsid by an as yet undetermined mechanism. TRIM5α acts prior to complete reverse transcription of viral RNA[96] and may also affect trafficking of the pre-integration complex[104].*

prior to nuclear import, although this function may not be required for restriction[4,104]. *In vitro* studies suggest that this block may occur at the level of uncoating of the viral capsid (Fig. 1), with accelerated uncoating and/or degradation of capsid being possible consequences of TRIM5α association[14,97]. Demonstration of direct binding of TRIM5α to capsid has proven difficult; however, there is compelling evidence that TRIM5α in cellular lysates can associate with detergent-stripped N-MLV virions[83] and also to recombinant HIV-1-derived CA-NC cores[97]. This interaction is proposed to lead to virion destabilization and disassembly by an unknown mechanism[14,74,97].

Several unique features of the B30.2/SPRY domain have made it the subject of intense interest and investigation as the determinant of species-specific restriction of primate lentiviruses. While this domain is certainly crucial for TRIM5α activity, several groups have carefully and painstakingly shown that the RING, B-box, and coiled-coil domains of TRIM5α are also necessary for optimal retroviral restriction[44,54,72,110]. Taken together, these studies suggest that the RBCC domain may function as a single, cohesive "effector domain" for TRIM5α protein function[18,72].

Found at the N-terminus of all TRIM family proteins, the RING domain has a zinc-binding motif often found in proteins with E3 ubiquitin ligase activity. Proteins with this domain can mediate auto-ubiquitination or transfer of ubiquitin to heterologous proteins[61]. TRIM5α is itself ubiquitinated, a modification that contributes to its rapid turnover by the proteasome[18,106]. Deletion of the RING domain as well point mutations affecting residues known to be critical for ubiquitin ligase activity modestly increase protein stability[18,96]. These changes diminish the ability of rhesus TRIM5α to inhibit HIV infection, although they do not abolish it completely[18]. Deletion of the RING domain prevents poly-ubiquitination of TRIM5α, suggesting that this domain is necessary for auto-ubiquitination[18]. Surprisingly, deletion of the RING domain or disruption of conserved residues has no effect on mono- and di-ubiquitination of TRIM5α[18], implying that while the RING domain may participate in poly-ubiquitination of self, other ubiquitin ligases may also modify TRIM5α. Recently, mono- and di-ubiquitination of proteins has been shown to influence protein function and subcellular localization in a manner that is independent of proteasomal degrada-

tion[16]. The persistence of mono- and di-ubiquitinated forms of TRIM5α raises the possibility that this modification influences protein function.

The B-box-2 domain follows the RING domain and is a critical component of the RBCC motif. While structures resembling the RING, coiled-coil and various C-terminal domains of TRIM family proteins are also found in other proteins, the B-box domain is found exclusively within this family[67,77]. TRIM family members can have either two consecutive B-box domains (B1 followed by B2), which differ in size and sequence, or a single B-box. Of the two types of B-boxes, a B-box-2 is found in all TRIM containing a single B-box domain[9,100]. Despite its high degree of conservation, the exact function of the B-box is unknown. Like the RING domain, the B-box is known to bind zinc[96,100]. B-box domains of other TRIM family members have been implicated in binding components of cell-signaling pathways[87,88]. The B-box is essential for retroviral restriction by rhesus TRIM5α, as deletion or disruption of this domain completely abolishes HIV-1 restriction[44,54,72,96].

The final domain comprising the canonical RBCC motif is the coiled-coil. This region is comprised of multiple α-helices involved in protein-protein interactions that mainly result in homo-multimers, but may mediate interaction with heterologous proteins[61,67,77]. Like many TRIM family members, overexpression of TRIM5α results in the formation of higher order structures, known as cytoplasmic bodies, although these structures are dispensable for restriction[73,77,91]. TRIM5α mutants lacking the coiled-coil domain fail to restrict viral infection[45,72]. *In vitro* cross-linking studies demonstrate that TRIM5α has a propensity for trimer formation that requires the coiled-coil domain alone[45,62]. It is believed that trimer formation orients the B30.2/SPRY domain for optimal target recognition[45,75,97]. However, ability to trimerize is not sufficient to mediate retroviral restriction by rhesus TRIM5α, as a construct in which the coiled-coil was replaced with a heterologous trimeric coiled-coil failed to inhibit HIV-1 infection despite efficient trimer formation and capsid interaction[45,54,62]. These results also imply that in addition to multimer formation, the coiled-coil domain may have additional attributes important for TRIM5α antiviral activity. The existence and significance of these interactions *in vivo* remains to be explored and could provide insight into the mechanism of TRIM5α-mediated retroviral restriction and regulation of TRIM family proteins in general.

The specificity and interspecies variability of viral restriction by TRIM5α lie within the C-terminal B30.2/SPRY domain. Sequence analysis has revealed considerable interspecies variability in the B30.2/SPRY domains of Old World and New World monkeys[64,81,92,98]. Variation in amino acid sequence in the B30.2/SPRY domains between different primate species accounts for much of the observed differential restriction of the various retroviruses tested (Table 1)[36,56,64,68,72,81,93,96,9]. Efforts to whittle down the sequence determinants for restriction specificity within the B30.2/SPRY domain by both genetic and molecular means have identified specific regions and even single amino acids that alter restriction activity when substitut or deleted. For example, substitution of R332 of human TRIM5α with a negatively-charged or non-charged amino acid is sufficient to allow restriction of HIV-1 without altering its ability to restrict N-MLV[55,110]. However, these and other studies also reinforce the notion that the entire B30.2/SPRY domain and surrounding regions are required for optimal retroviral restriction activity and specificity[36,55,64,68,72,80,110].

Several studies have shown quite convincingly that the B30.2/SPRY domain of TRIM5α is responsible for target recognition and that this target is a conformational ligand on the viral capsid[45,55,71,74,75,82,83,97]. In effect, the B30.2/SPRY domain is the pattern recognition molecule that identifies, engages and ultimately leads to destruction of susceptible virions by an unknown mechanism mediated by the RBCC domains. Based on comparisons with the molecular structures of SPRY-related domains from other proteins, the core of the TRIM5α B30.2/SPRY is thought to consist of two β-sheets sandwiched together to form a central hydrophobic core[33,60,68,103]. The residues forming this core are conserved among B30.2/SPRY domains from various proteins and are important for structural integrity of the scaffold[33,60,68,103]. Loops of variable length and containing non-conserved residues protrude out from the core structure to form a binding surface[68,103]. It is the organization of these loops that is thought to determine the characteristic variability and species specificity of ligand binding for TRIM5α[68,103,108]. The regions of variability among TRIM5α B30.2/SPRY domains for different species are located on these protruding loops[68,93,103]. Trimerization of TRIM5α may allow proper alignment of the binding surfaces to facilitate efficient binding to a symmetrical structural ligand on the viral capsid[45,52,53,62].

Natural history of the primate TRIM5 locus

Alignments and phylogenetic reconstructions of TRIM5α coding sequence reveal a locus with a very interesting but convoluted evolutionary past. It is prob-

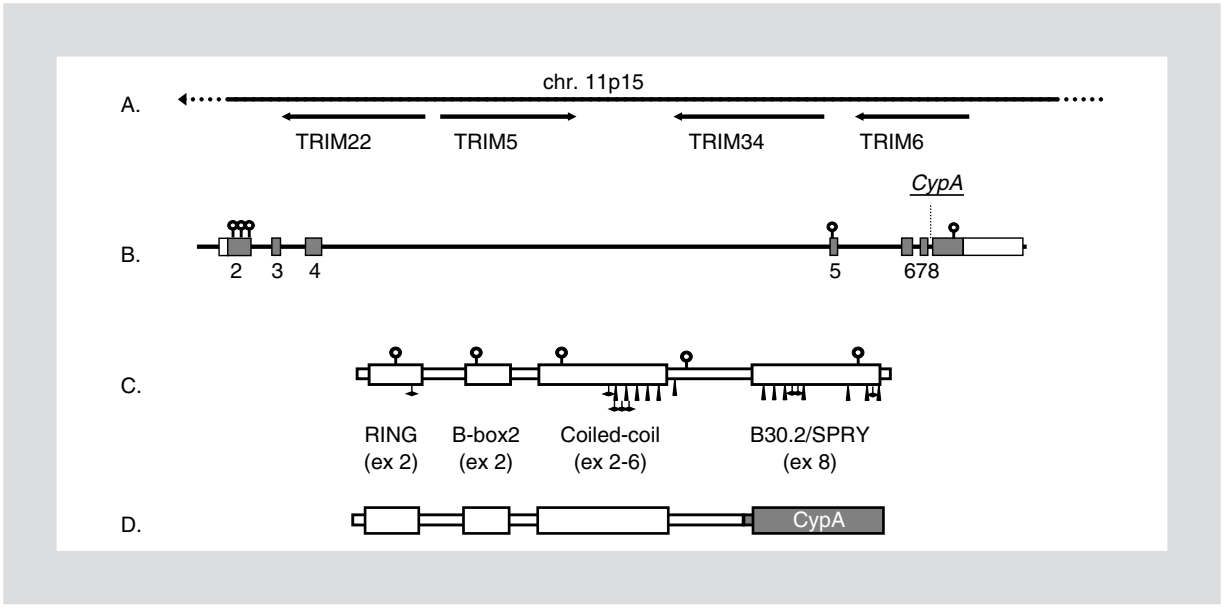

Figure 2. *The* TRIM5 *locus and TRIM5α.* **A***: schematic of human chromosomal region 11p15.4, depicting the cluster of TRIM loci containing* TRIM5, TRIM22, TRIM34 *and* TRIM6. *All four encode TRIM proteins with a B30.2/SPRY motif. A similar arrangement is found in the chimpanzee and rhesus macaque genomes (rhesus chr. 14)* **B:** *the* TRIM5 *gene. The coding sequence for the alpha isoform is spread across 7 exons, beginning with the RING domain in exon 2 and ending with the SPRY domain in exon 8. The lollipop structures indicate the approximate sites of five frequent nonsynonymous polymorphisms found in humans. The vertical, dashed line indicates the CypA integration site in owl monkey* TRIM5. **C:** *Cartoon depicting the TRIM5α protein. The RING, B-box and coiled-coil domains constitute the canonical RBCC "tripartite motif" found in all TRIM proteins (although some family members have two consecutive B-boxes). Lollipops above the protein indicate location of human nonsynonymous polymorphisms. For comparison, sites of frequent, nonsynonymous changes in sooty mangabeys (black flags) and rhesus macaques (black wedges) are indicated below the protein.* **D:** *schematic of the TRIMcyp fusion protein expressed in owl monkey cells. Because of a* CypA *sequence insertion between exons 7 and 8, owl monkeys express a fusion protein consisting of the RBCC domain of* TRIM5 *fused to cyclophilin A.*

able that the gene originally arose via duplication of another B30.2/SPRY-encoding *TRIM* gene. *TRIM5* is located on chromosome 11 (human numbering) in a cluster that also includes *TRIM6, TRIM22* and *TRIM34,* all of which encode B30.2/SPRY domains (Fig. 2). These four genes group together in a phylogenetic tree incorporating multiple, B30.2/SPRY-type *TRIM* loci[53,89], consistent with a common origin and/or a history of concerted evolution.

TRIM5 has suffered a variety of molecular alterations at different times and in different places during primate evolution, and the gene displays a high degree of sequence divergence between primate species. Changes include deletions, insertions and extensive nucleotide substitutions, as well as occasional serial duplications of short stretches of sequence within the B30.2/SPRY domain[56,65,69,80,81,92,93]. Certain subdomains bear the distinctive hallmarks of positive selection, with the greatest level of amino-acid variation found in the B30.2/SPRY domain[56,69,81,92]. Sequence and length variation in this domain is particularly concentrated in four subregions (referred to as V1 through V4)[92]. Despite the high degree of variation, phylogenetic trees constructed from aligned orthologous TRIM5α sequences generally recapitulate the well-established phylogeny of primates, particularly internal nodes separating the major divisions (apes, Old World monkeys and New World monkeys)[65,68,69,81,92].

Sawyer, et al.[81] detected the footprints of intensive positive selection operating on primate TRIM5α, particularly in a small cluster of codons within the B30.2/SPRY domain of hominids (humans and the related great apes). They were then able to back up phylogenetic prediction by proving that this patch of sequence functioned as a key determinant of differential target-specificity in tissue-culture experiments[81,107]. This fits nicely with an evolutionary scenario wherein amino-acid variation between TRIM5α proteins derived from different lineages reflects a differential history of positive selection, presumably due to past infections with pathogenic retroviruses. The pattern is reminiscent of major histocompatibility complex (MHC) loci, in which codons under strong positive selection map to the peptide-binding regions[40,41]. In the case of TRIM5α, selection seems to be focused on the B30.2/SPRY domain, and a variety of studies now indicate that the B30.2/SPRY

domain is the probable interface with incoming viral capsids. Experimental mapping of specificity determinants by other groups were also consistent with the Sawyer, et al. predictions[72,98,110]. Importantly, the signal for positive selection is widespread among both Old and New World primate lineages, suggesting that TRIM5α has a rich history of countering viral assaults on the ancestors of modern species. It is also indirect testimony to the fact that episodic retroviral epidemics, in some ways analogous to the modern AIDS epidemic, have been part and parcel of primate evolution for tens of millions of years.

Polymorphism

To date, extensive investigation of within-species variation (polymorphism) in *TRIM5* has only been reported for humans[32,43,80,94] and for two species of Old World monkey[65]. Reports of human *TRIM5* polymorphism were based on sequence data collected from HIV/AIDS cohorts and from human genomic DNA diversity collections, as well as human SNP databases. Altogether, *TRIM5* genotype data from more than a thousand individual human samples have been reported, and a handful of common polymorphisms have been described (Fig. 2). Sawyer, et al.[80] amplified and sequenced the *TRIM5* locus from a human diversity panel consisting of genomic DNA samples from 37 geographically and ethnically distinct individuals; the analysis included the entire coding sequence as well as five out of the six intervening introns. Twenty SNP were identified, including six nonsynonymous changes with minor allele frequencies greater than 5%. These were located in the RING domain *(H43Y)*, the B-box-2 domain *(V112F)*, in or near the predicted coiled-coil region *(R136Q, R238W, G249D)*, and in the B30.2/SPRY domain *(H419Y).* Five of these *(H43Y, V112F, R136Q, G249D* and *H419Y)* were also found by others during screening of genomic DNA samples from multiple HIV/AIDS cohorts[32,43,94]. Thus far, the *238W* variant has only been reported among Biaka Pygmy individuals[80]. A number of less common alleles have also been described, including additional, nonsynonymous SNP[43,94] and multiple SNP in untranslated regulatory regions and introns[43,80,94].

Remarkably, nonsynonymous polymorphisms have not been reported in the variable regions of the human TRIM5α B30.2/SPRY domain. It has been suggested that the overall low-level of polymorphism in the 3' half of the gene could be the result of a selective sweep, perhaps in the form of an ancient retroviral epidemic[80]. If true, by wiping out variation in the human B30.2/SPRY domain, this prehistoric episode may have contributed to the magnitude of the current AIDS pandemic. Further insight into the recent history of human TRIM5α should be gained possibly by comparing variation in humans and chimpanzees, particularly by including intron sequences, which generally evolve in neutral fashion, in the analysis. Currently, a few chimpanzee *TRIM5* sequences are available in the public databases, but to our knowledge extensive sampling of individual chimpanzees has not been reported.

Immortalized human B-cell lines (BLCL) from individuals heterozygous or homozygous for *43Y* demonstrated significantly reduced capacity to restrict N-tropic MLV, and recombinant, human TRIM5α vectors incorporating the H-to-Y change at position 43 are similarly defective in tissue-culture based assays[32,43,80]. The major allele *(H43)* does not appreciably restrict HIV-1, so additional effects of the H43Y mutation on HIV-1 infection may not always be apparent. For example, Speelmon, et al.[94] did not detect a significant difference in susceptibility to single-cycle HIV-1 infection of CD4+ T-cells from *43H/H* versus *43Y/Y* individuals. While TRIM5α does not appear to have a significant impact on HIV-1 infection of humans, it is noteworthy that the defective *43Y* allele is present at high frequency in human populations and could therefore play a role in susceptibility to other retroviral pathogens[80]. Emerman[21] has also pointed out that the frequency of *43Y* in human populations argues against TRIM5 encoding an essential cellular function separate from viral restriction.

Another common polymorphism in humans occurs at residue 136 in the coiled-coil domain *(R136Q).* At least one study reported that introduction of a glutamine at position 136 of human TRIM5α by site-directed mutagenesis resulted in slightly more effective restriction of HIV-1[43], although a different study failed to detect a difference[32]. A possible protective effect of the *136Q* allele has also been reported (see below).

Thus far, association studies have uncovered little-or-no effect of *TRIM5* genotype on disease progression in HIV-1 infected individuals, and have only hinted at possible correlations between *TRIM5* polymorphism and risk of acquiring HIV-1 infection[32,43,94]. The first such study, reported by Speelmon, et al.[94], compared allele frequencies between HIV-1-infected and HIV-1-exposed, seronegative individuals in a cohort consisting predominantly of men with European-American ancestry. A significant association between one of nine common haplotypes and HIV-1 infection status was found in this

study, although no statistically significant correlation between individual SNP and susceptibility was revealed.

More recently, Javanbakht, et al.[43] reported a very thorough analysis combining new *TRIM5* SNP discovery and association studies involving multiple HIV/AIDS cohorts. Proportional hazards regression found no significant associations between individual *TRIM5* polymorphisms and three different definitions of AIDS/progression. In this study, a possible protective effect was detected for one haplotype, and unlinked alleles of four SNP were also weakly associated with increased or decreased risk of infection in African Americans but not in European Americans. A potentially protective allele (SNP 136Q) identified in this study[43] was also present in a haplotype associated with increased risk in the Speelmon, et al.[94] study. Curiously, this discrepancy also extended to *in vitro* restriction assays: while the Javanbakht, et al. study found that recombinant TRIM5α with a Q at position 136 was modestly better than wild-type at restricting single-cycle HIV-1 in HeLa cells, the Speelmon et al. study found no difference in susceptibility of primary CD4+ T-cells from *136Q/Q* and *136R/R* patients to single-cycle HIV-1 infection.

It is noteworthy that these studies did not converge on the same answer, that the reported associations were modest at best, and that some specific observations did not maintain significance after correction for multiple tests and/or false discovery[32,43,95]. The lack of concordance could be related to obvious differences between the studies: in addition to different approaches to data analysis, the cohorts analyzed in each study differed in size and composition, and different measures of AIDS/progression were used. Finally, it may be that some or all of the reported results represent random, chance associations, in which case they should not hold up if larger, more extensive surveys are conducted.

Newman, et al.[65] characterized polymorphic variation in a small number of unrelated Asian macaques (rhesus macaques, *M. mulatta*) and African monkeys (sooty mangabeys, *C. atys*). The pattern of polymorphism was similar in both species, with frequent changes clustered in a 50-residue stretch of the coiled-coil domain and in the variable portion of the B30.2/SPRY domain. This is in striking contrast to the situation in humans, where none of the reported polymorphisms lie within the variable regions of the B30.2/SPRY domain (Fig. 2), and a search of the current human SNP database (dbSNP) reveals numerous, low frequency SNP throughout the gene but none that map to this region[32,43,80,94].

Interestingly, some polymorphisms were shared by the two species of monkeys, suggesting that the ancestral alleles coexisted in a common progenitor of rhesus macaques and sooty mangabeys, and have persisted together in both lineages until the present day – a span of at least eight million years. Among the polymorphisms shared by rhesus macaques and sooty mangabeys was a P/Q polymorphism at position 334 in the B30.2/SPRY domain[35,65]. Residue 334 (332 in human TRIM5α) is also variable between species (typically a P, Q, or R) and is known to influence target-specificity. The presence of ancient, shared polymorphism runs contrary to the expectations of random genetic drift, and raises the possibility that balancing selection has also played a role in the evolution of TRIM5α, at least in these species.

TRIM5CypA

For several years, researchers were puzzled by the observation that owl monkey cell lines restrict HIV-1 infection, and moreover, by the finding that this restriction could be ameliorated by treatment of the cells with the CypA binding protein CsA[102]. This observation was initially confusing, because CypA was known to be a positive-acting cofactor for HIV-1 replication in human cells, and treatment of human cell lines with CsA normally delayed HIV-1 replication[10-12,22,23,58,99]. Independently of the original discovery of TRIM5α-mediated restriction, Sayah, et al.[82] cloned a cDNA encoding a TRIM5-CypA fusion protein from owl monkeys. The fusion protein was also identified in a screen for TRIM5-related transcripts in owl monkey cells[66]. The predicted protein encoded the RBCC motif of TRIM5α, but contained a CypA domain in place of the B30.2/SPRY domain. Inspection of the owl monkey locus revealed that the fusion was generated by retrotranspositional insertion of a CypA open reading frame between exon 7 and exon 8 of *TRIM5* (exon 8 encodes the B30.2/SPRY domain) (Fig. 2). The existence of this protein provided a ready explanation for CsA-sensitive restriction of HIV-1 in owl monkey cells, and indirectly provided insight into the relationship between structure and function of TRIM5α itself. Because TRIM family members can be subdivided according to the nature of the polypeptide comprising the C-terminal domain, the existence of TRIMCyp suggests that retrotranspositional insertion is a mechanism by which some novel TRIM protein family members may arise[105].

Ribeiro, et al.[78] screened multiple individuals representing each of several genera of New World monkeys,

including multiple species from the genus *Aotus* (owl monkeys), for the presence of the *TRIMCyp* variant. They found that the retrotransposed *CypA* insertion is unique to species of the *Aotus* genus, and that *TRIMCyp* orthologues cluster with an owl monkey *CypA* sequence in a phylogenetic tree incorporating *CypA* sequences from multiple primate lineages. These results are consistent with a single retrotransposition event having occurred in a common ancestor of all extant *Aotus* species sometime between 4.5-million and 22-million years ago[78]. Although there are currently no data to support (or reject) the hypothesis, it is tempting to imagine that the *TRIMCyp* allele was selected, and ultimately driven to fixation in the owl monkey lineage, by virtue of its ability to restrict some unknown (and possibly extinct) retroviral pathogen.

A bovine TRIM with antiretroviral activity

Recently, two groups reported characterization of a TRIM-family protein from cows with the capacity to restrict multiple retroviruses[89,111]. Both studies were based on previous reports that a bovine cell line expressed a post-entry block to replication of multiple retroviruses[6,42,79,101], and in both cases a bioinformatics approach led to the identification of expressed sequence tags (EST) predicted to encode a TRIM protein with a B30.2/SPRY domain. Inhibiting expression of the putative factor by siRNA alleviated restriction of infection by multiple retroviruses[89,111]. In tissue-culture based assays, the bovine protein restricted infection by HIV-1, HIV-2, SIVmac, FIV (feline immunodeficiency virus) and N-MLV. Based on sequence alignments and phylogenetic analysis, Si, et al.[89] have proposed that antiretroviral activity of this bovine TRIM and TRIM5α represent a compelling case for convergent evolution, although it is formally possible that antiretroviral activity first appeared in an ancestral *TRIM* that gave rise to both genes. Final resolution of the phylogenetic relationship between these two genes will require detailed comparison of the loci in their respective chromosomal contexts, including flanking sequences and introns.

Conclusions

There are now several examples in the TRIM5α literature demonstrating restriction of the same retrovirus by cells derived from different primate species. Moreover, a number of primate TRIM5α orthologues have been shown to retain restriction activity when expressed in heterologous cell lines. Similarly, the recently described bovine TRIM restricts a variety of retroviruses even when expressed in canine or feline cells[89,111]. These observations imply that cofactors involved in TRIM5α-mediated restriction, if they exist, should be fairly well conserved (at least among mammals). However, most published results are based on overexpression of TRIM5α, which could obscure the effects of cellular context or the presence of cofactors with subtle and/or regulatory roles in restriction (for example, see discussion in reference[79]). Thus far, published reports have also not ruled out the possibility of downstream consequences of the TRIM5α-CA interaction, such as recruitment of additional factors, trafficking of the TRIM5α-CA complex, or signaling to other components of innate or adaptive immunity.

The human TRIM5α protein is not a globally defective protein, as it potently restricts N-MLV and, when overexpressed, has some discernable activity against HIV-1. This opens the door for possible therapeutic intervention if small molecules can be identified that function to enhance human TRIM5α activity against HIV-1. Unlike traditional antiviral screens, which seek to identify inhibitors of viral replication, candidate molecules in this case are expected to provide what is essentially a gain-of-function. While such a strategy is atypical, it is not without precedent[34].

Currently, there are no feasible animal model systems that permit full replication of HIV-1 and which mimic persistent HIV-1 infection in human AIDS patients. Identifying the genetic barriers to HIV replication has implications for developing such models. For example, understanding the nature of the barriers provides a strategy for surmounting those blocks through the construction of resistant HIV-1 clones. At least two groups have recently reported progress along these lines, using similar strategies based on construction of recombinant SIV/HIV-1 clones and limited adaptation in cultured monkey cells[37,46]. Given the high degree of interspecies variation in TRIM5 sequence and specificity, and the possibility of genetic and phenotypic polymorphism within species, additional opportunities may be found by exploiting the underlying population-genetics of restriction. For example, it may be possible to identify polymorphic variants of *TRIM5* in nonhuman primates that would be permissive for HIV-1 infection. There is a small but intriguing literature describing transient, abortive infection of pig-tail macaques, including evidence for adaptive immune responses, after experimental inoculation with HIV-1[1,2,28,48,49,70]. Hypothetically, a suboptimal match between a common al-

lele of pig-tail macaque TRIM5α and the HIV-1 capsid could explain such observations. This would be consistent with the timing of TRIM5α-mediated restriction, which blocks the early stage of the replication cycle. A defective TRIM5α would permit infection of pig-tail macaque cells, which would then produce virus and induce adaptive immune responses, but spreading infection would still be constrained by the presence of additional, downstream restrictions, such as that imposed by APOBEC3G[30,31]. Variation in pig-tail macaque TRIM5α has been observed[35].

Retroviruses are widespread in nature, and the sheer abundance of endogenous proviruses in vertebrate genomes is indisputable proof that retroviruses have been colonizing animal hosts for hundreds of millions of years[29]. Under such circumstances, the convergence of the completely unrelated Fv-1 and TRIM5 proteins on the early post-entry step of retroviral replication is perhaps not all that surprising. That such loci were first identified in mice and humans simply reflects the priorities of biomedical research, and it is quite possible that similar adaptations arose many times during the course of metazoan evolution and await discovery. In this regard, it is also worth noting that TRIM5α experiments reported to date have focused overwhelmingly on lentiviruses (HIV-1, HIV-2, various SIV, EIAV, FIV) and a single gammaretrovirus (MLV) (Table 1). There are very few reports in which representatives of other retroviral genera have been tested for susceptibility to TRIM5α-mediated restriction[50,65]. Thus, it is not clear that TRIM5α restricts other retroviruses, let alone members of other virus families. It is also possible, perhaps even likely, that unique restriction mechanisms exist in nature that specifically target members of other retroviral genera.

References

1. Agy M, Frumkin L, Corey L, et al. Infection of Macaca nemestrina by HIV-1. Science 1992;257:103-6.
2. Agy M, Schmidt A, Florey M, et al. Serial *in vivo* passage of HIV-1 infection in Macaca nemestrina. Virology 1997;238:336-43.
3. Alter H, Eichberg J, Masur H, et al. Transmission of HTLV-III infection from human plasma to chimpanzees: an animal model for AIDS. Science 1984;226:549-52.
4. Anderson J, Campbell E, Wu X, Vandegraaff N, Engelman A, Hope T. Proteasome inhibition reveals that a functional preintegration complex intermediate can be generated during restriction by diverse TRIM5 proteins. J Virol 2006;80:9754-60.
5. Besnier C, Takeuchi Y, Towers G. Restriction of lentivirus in monkeys. Proc Natl Acad Sci USA 2002;99:11920-5.
6. Besnier C, Ylinen L, Strange B, et al. Characterization of murine leukemia virus restriction in mammals. J Virol 2003;77:13403-6.
7. Bieniasz P. Intrinsic immunity: a front-line defense against viral attack. Nat Immunol 2004;5:1109-15.
8. Bieniasz P. Restriction factors: a defense against retroviral infection. Trends Microbiol 2003;11:286-91.
9. Borden K, Lally J, Martin S, O'Reilly N, Etkin L, Freemont P. Novel topology of a zinc-binding domain from a protein involved in regulating early Xenopus development. Embo J 1995;14:5947-56.
10. Braaten D, Aberham C, Franke E, Yin L, Phares W, Luban J. Cyclosporine A-resistant HIV-1 mutants demonstrate that Gag encodes the functional target of cyclophilin A. J Virol 1996;70:5170-6.
11. Braaten D, Franke E, Luban J. Cyclophilin A is required for an early step in the life cycle of HIV-1 before the initiation of reverse transcription. J Virol 1996;70:3551-60.
12. Braaten D, Luban J. Cyclophilin A regulates HIV-1 infectivity, as demonstrated by gene targeting in human T cells. Embo J 2001;20:1300-9.
13. Bukovsky A, Weimann A, Accola M, Gottlinger H. Transfer of the HIV-1 cyclophilin-binding site to SIV from Macaca mulatta can confer both cyclosporin sensitivity and cyclosporin dependence. Proc Natl Acad Sci USA 1997;94:10943-8.
14. Chatterji U, Bobardt M, Gaskill P, Sheeter D, Fox H, Gallay P. Trim5α accelerates degradation of cytosolic capsid associated with productive HIV-1 entry. J Biol Chem 2006;281:37025-33.
15. Cowan S, Hatziioannou T, Cunningham T, Muesing M, Gottlinger H, Bieniasz P. Cellular inhibitors with Fv1-like activity restrict human and simian immunodeficiency virus tropism. Proc Natl Acad Sci USA 2002;99:11914-9.
16. d'Azzo A, Bongiovanni A, Nastasi T. E3 ubiquitin ligases as regulators of membrane protein trafficking and degradation. Traffic 2005;6:429-41.
17. Daniel M, Letvin N, King N, et al. Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. Science 1985;228:1201-4.
18. Diaz-Griffero F, Li X, Javanbakht H, et al. Rapid turnover and polyubiquitylation of the retroviral restriction factor TRIM5. Virology 2006;349:300-15.
19. Dorfman T, Gottlinger H. The HIV-1 capsid p2 domain confers sensitivity to the cyclophilin-binding drug SDZ NIM 811. J Virol 1996;70:5751-7.
20. Dorfman T, Weimann A, Borsetti A, Walsh C, Gottlinger H. Active-site residues of cyclophilin A are crucial for its incorporation into HIV-1 virions. J Virol 1997;71:7110-3.
21. Emerman M. How TRIM5α defends against retroviral invasions. Proc Natl Acad Sci USA 2006;103:5249-50.
22. Franke E, Luban J. Inhibition of HIV-1 replication by cyclosporine A or related compounds correlates with the ability to disrupt the Gag-cyclophilin A interaction. Virology 1996;222:279-82.
23. Franke E, Yuan H, Luban J. Specific incorporation of cyclophilin A into HIV-1 virions. Nature 1994;372:359-62.
24. Fultz P, McClure H, Daugharty H, et al. Vaginal transmission of HIV to a chimpanzee. J Infect Dis 1986;154:896-900.
25. Gajdusek D, Amyx H, Gibbs Jr. C, et al. Infection of chimpanzees by human T-lymphotropic retroviruses in brain and other tissues from AIDS patients. Lancet 1985;1:55-6.
26. Gardner M. Simian AIDS: an historical perspective. J Med Primatol 2003;32:180-6.
27. Gardner M, Luciw P. Animal models of AIDS. Faseb J 1989;3:2593-606.
28. Gartner S, Liu Y, Lewis M, et al. HIV-1 infection in pigtailed macaques. AIDS Res Hum Retroviruses 1994;10(Suppl 2):S129-33.
29. Gifford R, Tristem M. The evolution, distribution and diversity of endogenous retroviruses. Virus Genes 2003;26:291-315.
30. Goff S. Genetic control of retrovirus susceptibility in mammalian cells. Annu Rev Genet 2004;38:61-85.
31. Goff S. Retrovirus restriction factors. Mol Cell 2004;16:849-59.
32. Goldschmidt V, Bleiber G, May M, Martinez R, Ortiz M, Telenti A. Role of common human TRIM5α variants in HIV-1 disease progression. Retrovirology 2006;3:54.
33. Grutter C, Briand C, Capitani G, et al. Structure of the PRYSPRY-domain: implications for autoinflammatory diseases. FEBS Lett 2006;580:99-106.
34. Guo Z, Zhou D, Schultz P. Designing small-molecule switches for protein-protein interactions. Science 2003;288:2042-5.
35. Hall L, Newman R, Pery E, Farzan M, Johnson W. 2007 [unpublished observations].

36. Hatziioannou T, Perez-Caballero D, Yang A, Cowan S, Bieniasz P. Retrovirus resistance factors Ref1 and Lv1 are species-specific variants of TRIM5α. Proc Natl Acad Sci USA 2004;101:10774-9.
37. Hatziioannou T, Princiotta M, Piatak Jr. M, et al. Generation of simian-tropic HIV-1 by restriction factor evasion. Science 2006;314:95.
38. Himathongkham S, Luciw P. Restriction of HIV-1 (subtype B) replication at the entry step in rhesus macaque cells. Virology 1996;219:485-8.
39. Hofmann W, Schubert D, LaBonte J, et al. Species-specific, postentry barriers to primate immunodeficiency virus infection. J Virol 1999;73:10020-8.
40. Hughes A, Nei M. Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. Nature 1988;335:167-70.
41. Hughes A, Yeager M. Natural selection at major histocompatibility complex loci of vertebrates. Annu Rev Genet 1998;32:415-35.
42. Ikeda Y, Collins M, Radcliffe P, Mitrophanous K, Takeuchi Y. Gene transduction efficiency in cells of different species by HIV and EIAV vectors. Gene Ther 2002;9:932-8.
43. Javanbakht H, An P, Gold B, et al. Effects of human TRIM5α polymorphisms on antiretroviral function and susceptibility to HIV infection. Virology 2006;354:15-27.
44. Javanbakht H, Diaz-Griffero F, Stremlau M, Si Z, Sodroski J. The contribution of RING and B-box 2 domains to retroviral restriction mediated by monkey TRIM5α. J Biol Chem 2005;280:26933-40.
45. Javanbakht H, Yuan W, Yeung D, et al. Characterization of TRIM5α trimerization and its contribution to HIV capsid binding. Virology 2006;353:234-46.
46. Kamada K, Igarashi T, Martin M, et al. Generation of HIV-1 derivatives that productively infect macaque monkey lymphoid cells. Proc Natl Acad Sci USA 2006;103:16959-64.
47. Keckesova Z, Ylinen L, Towers G. The human and African green monkey TRIM5α genes encode Ref1 and Lv1 retroviral restriction factor activities. Proc Natl Acad Sci USA 2004101:10780-5.
48. Kent S, Corey L, Agy M, Morton W, McElrath M, Greenberg P. Cytotoxic and proliferative T cell responses in HIV-1-infected Macaca nemestrina. J Clin Invest 1995;95:248-56.
49. Kimball L, Bosch M. In vitro HIV-1 infection in Macaca nemestrina PBMCs is blocked at a step beyond reverse transcription. J Med Primatol 1998;27:99-103.
50. Lee Y, Bieniasz P. Reconstitution of an infectious human endogenous retrovirus. PLoS Pathog 2007;3:e10.
51. Li J, Lord C, Haseltine W, Letvin N, Sodroski J. Infection of cynomolgus monkeys with a chimeric HIV-1/SIVmac virus that expresses the HIV-1 envelope glycoproteins. J Acquir Immune Defic Syndr 1992;5:639-46.
52. Li S, Hill C, Sundquist W, Finch J. Image reconstructions of helical assemblies of the HIV-1 CA protein. Nature 2003;407:409-13.
53. Li X, Gold B, O'Huigin C, et al. Unique features of TRIM5α among closely related human TRIM family members. Virology 2007;360: 419-33.
54. Li X, Li Y, Stremlau M, et al. Functional replacement of the RING, B-Box 2, and coiled-coil domains of tripartite motif 5α (TRIM5α) by heterologous TRIM domains. J Virol 2006;80:6198-206.
55. Li Y, Li X, Stremlau M, Lee M, Sodroski J. Removal of arginine 332 allows human TRIM5alpha to bind HIV capsids and to restrict infection. J Virol 2006;80:6738-44.
56. Liu H, Wang Y, Liao C, Kuang Y, Zheng Y, Su B. Adaptive evolution of primate TRIM5α, a gene restricting HIV-1 infection. Gene 2005;362:109-16.
57. Luban J. Cyclophilin A, TRIM5, and resistance to HIV-1 infection. J Virol 2007;81:1054-61.
58. Luban J, Bossolt K, Franke E, Kalpana G, Goff S. HIV-1 Gag protein binds to cyclophilins A and B. Cell 1993;73:1067-78.
59. Lusso P, Markham P, Ranki A, et al. Cell-mediated immune response toward viral envelope and core antigens in gibbon apes (Hylobates lar) chronically infected with HIV-1. J Immunol 1988;141:2467-73.
60. Masters S, Yao S, Willson T, et al. The SPRY domain of SSB-2 adopts a novel fold that presents conserved Par-4-binding residues. Nat Struct Mol Biol 2006;13:77-84.
61. Meroni G, Diez-Roux G. TRIM/RBCC, a novel class of 'single protein RING finger' E3 ubiquitin ligases. Bioessays 2005;27:1147-57.
62. Mische C, Javanbakht H, Song B, et al. Retroviral restriction factor TRIM5α is a trimer. J Virol 2005;79:14446-50.
63. Munk C, Brandt S, Lucero G, Landau N. A dominant block to HIV-1 replication at reverse transcription in simian cells. Proc Natl Acad Sci USA 2002;99:13843-8.
64. Nakayama E, Miyoshi H, Nagai Y, Shioda T. A specific region of 37 amino acid residues in the SPRY (B30.2) domain of African green monkey TRIM5α determines species-specific restriction of SIVmac infection. J Virol 2005;79:8870-7.
65. Newman R, Hall L, Connole M, et al. Balancing selection and the evolution of functional polymorphism in Old World monkey TRIM5α. Proc Natl Acad Sci USA 2006;103:19134-9.
66. Nisole S, Lynch C, Stoye J, Yap M. A Trim5-cyclophilin A fusion protein found in owl monkey kidney cells can restrict HIV-1. Proc Natl Acad Sci USA 2004;101:13324-8.
67. Nisole S, Stoye J, Saib A. TRIM family proteins: retroviral restriction and antiviral defence. Nat Rev Microbiol 2005;3:799-808.
68. Ohkura S, Yap M, Sheldon T, Stoye J. All three variable regions of the TRIM5α B30.2 domain can contribute to the specificity of retrovirus restriction. J Virol 2006;80:8554-65.
69. Ortiz M, Bleiber G, Martinez R, Kaessmann H, Telenti A. Patterns of evolution of host proteins involved in retroviral pathogenesis. Retrovirology 2006;3:11.
70. Otten R, Brown B, Simon M, et al. Differential replication and pathogenic effects of HIV-1 and HIV-2 in Macaca nemestrina. Aids 1994;8:297-306.
71. Owens C, Song B, Perron M, Yang P, Stremlau M, Sodroski J. Binding and susceptibility to postentry restriction factors in monkey cells are specified by distinct regions of the HIV-1 capsid. J Virol 2004;78:5423-37.
72. Perez-Caballero D, Hatziioannou T, Yang A, Cowan S, Bieniasz P. Human tripartite motif 5α domains responsible for retrovirus restriction activity and specificity. J Virol 2005;79:8969-78.
73. Perez-Caballero D, Hatziioannou T, Zhang F, Cowan S, Bieniasz P. Restriction of HIV-1 by TRIM-CypA occurs with rapid kinetics and independently of cytoplasmic bodies, ubiquitin, and proteasome activity. J Virol 2005;79:15567-72.
74. Perron M, Stremlau M, Lee M, Javanbakht H, Song B, Sodroski J. The human TRIM5α restriction factor mediates accelerated uncoating of the N-tropic murine leukemia virus capsid. J Virol 2007;81:2138-48.
75. Perron M, Stremlau M, Sodroski J. Two surface-exposed elements of the B30.2/SPRY domain as potency determinants of N-tropic murine leukemia virus restriction by human TRIM5α. J Virol 2006;80:5631-6.
76. Perron M, Stremlau M, Song B, Ulm W, Mulligan R, Sodroski J. TRIM5α mediates the postentry block to N-tropic murine leukemia viruses in human cells. Proc Natl Acad Sci USA 2004;101: 11827-32.
77. Reymond A, Meroni G, Fantozzi A, et al. The tripartite motif family identifies cell compartments. Embo J 2001;20:2140-51.
78. Ribeiro I, Menezes A, Moreira M, Bonvicino C, Seuanez H, Soares M. Evolution of cyclophilin A and TRIMCyp retrotransposition in New World primates. J Virol 2005;79:14998-5003.
79. Saenz D, Teo W, Olsen J, Poeschla E. Restriction of feline immunodeficiency virus by Ref1, Lv1, and primate TRIM5α proteins. J Virol 2005;79:15175-88.
80. Sawyer S, Wu L, Akey J, Emerman M, Malik H. High-frequency persistence of an impaired allele of the retroviral defense gene TRIM5α in humans. Curr Biol 2006;16:95-100.
81. Sawyer S, Wu L, Emerman M, Malik H. Positive selection of primate TRIM5α identifies a critical species-specific retroviral restriction domain. Proc Natl Acad Sci USA 2005;102:2832-7.
82. Sayah D, Sokolskaja E, Berthoux L, Luban J. Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. Nature 2004;430:569-73.
83. Sebastian S, Luban J. TRIM5α selectively binds a restriction-sensitive retroviral capsid. Retrovirology 2005;2:40.

84. Shibata R, Adachi A. SIV/HIV recombinants and their use in studying biological properties. AIDS Res Hum Retroviruses 1992; 8:403-9.
85. Shibata R, Kawamura M, Sakai H, Hayami M, Ishimoto A, Adachi A. Generation of a chimeric human and simian immunodeficiency virus infectious to monkey peripheral blood mononuclear cells. J Virol 1991;65:3514-20.
86. Shibata R, Sakai H, Kawamura M, Tokunaga K, Adachi A. Early replication block of HIV-1 in monkey cells. J Gen Virol 1995;76:2723-30.
87. Shoham N, Cohen L, Gazit A, Yaniv A. The Tat protein of the caprine arthritis encephalitis virus interacts with the Notch2 EGF-like repeats and the epithelin/granulin precursor. Intervirology 2003;46:239-44.
88. Short K, Hopwood B, Yi Z, Cox T. MID1 and MID2 homo- and heterodimerise to tether the rapamycin-sensitive PP2A regulatory subunit, alpha 4, to microtubules: implications for the clinical variability of X-linked Opitz GBBB syndrome and other developmental disorders. BMC Cell Biol 2002;3:1.
89. Si Z, Vandegraaff N, O'Huigin C, et al. Evolution of a cytoplasmic tripartite motif (TRIM) protein in cows that restricts retroviral infection. Proc Natl Acad Sci USA 2006;103:7454-9.
90. Sokolskaja E, Luban J. Cyclophilin, TRIM5, and innate immunity to HIV-1. Curr Opin Microbiol 2006;9:404-8.
91. Song B, Diaz-Griffero F, Park do H, Rogers T, Stremlau M, Sodroski J. TRIM5α association with cytoplasmic bodies is not required for antiretroviral activity. Virology 2005;343:201-11.
92. Song B, Gold B, O'Huigin C, et al. The B30.2(SPRY) domain of the retroviral restriction factor TRIM5α exhibits lineage-specific length and sequence variation in primates. J Virol 2005;79:6111-21.
93. Song B, Javanbakht H, Perron M, Park D, Stremlau M, Sodroski J. Retrovirus restriction by TRIM5α variants from Old World and New World primates. J Virol 2005;79:3930-7.
94. Speelmon E, Livingston-Rosanoff D, Li S, et al. Genetic association of the antiviral restriction factor TRIM5α with HIV-1 infection. J Virol 2006;80:2463-71.
95. Storey J, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci USA 2003;100:9440-5.
96. Stremlau M, Owens C, Perron M, Kiessling M, Autissier P, Sodroski J. The cytoplasmic body component TRIM5α restricts HIV-1 infection in Old World monkeys. Nature 2004;427:848-53.
97. Stremlau M, Perron M, Lee M, et al. Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5α restriction factor. Proc Natl Acad Sci USA 2006;103:5514-9.
98. Stremlau M, Perron M, Welikala S, Sodroski J. Species-specific variation in the B30.2(SPRY) domain of TRIM5α determines the potency of HIV restriction. J Virol 2005;79:3139-45.
99. Thali M, Bukovsky A, Kondo E, et al. Functional association of cyclophilin A with HIV-1 virions. Nature 1994;372:363-5.
100. Torok M, Etkin L. Two B or not two B? Overview of the rapidly expanding B-box family of proteins. Differentiation 2001;67:63-71.
101. Towers G, Bock M, Martin S, Takeuchi Y, Stoye J, Danos O. A conserved mechanism of retrovirus restriction in mammals. Proc Natl Acad Sci USA 2000;97:12295-9.
102. Towers G, Hatziioannou T, Cowan S, Goff S, Luban J, Bieniasz P. Cyclophilin A modulates the sensitivity of HIV-1 to host restriction factors. Nat Med 2003;9:1138-43.
103. Woo J, Imm J, Min C, Kim K, Cha S, Oh B. Structural and functional insights into the B30.2/SPRY domain. Embo J 2006; 25:1353-63.
104. Wu X, Anderson J, Campbell E, Joseph A, Hope T. Proteasome inhibitors uncouple rhesus TRIM5α restriction of HIV-1 reverse transcription and infection. Proc Natl Acad Sci USA 2006;103: 7465-70.
105. Xing J, Wang H, Belancio V, Cordaux R, Deininger P, Batzer M. Emergence of primate genes by retrotransposon-mediated sequence transduction. Proc Natl Acad Sci USA 2006;103: 17608-13.
106. Xu L, Yang L, Moitra P, et al. BTBD1 and BTBD2 colocalize to cytoplasmic bodies with the RBCC/tripartite motif protein, TRIM5delta. Exp Cell Res 2003;288:84-93.
107. Yang Z. The power of phylogenetic comparison in revealing protein function. Proc Natl Acad Sci USA 2005;102:3179-80.
108. Yao S, Liu M, Masters S, et al. Dynamics of the SPRY domain-containing SOCS box protein 2: flexibility of key functional loops. Protein Sci 2006;15:2761-72.
109. Yap M, Nisole S, Lynch C, Stoye J. Trim5α protein restricts both HIV-1 and murine leukemia virus. Proc Natl Acad Sci USA 2004; 101:10786-91.
110. Yap M, Nisole S, Stoye J. A single amino acid change in the SPRY domain of human Trim5α leads to HIV-1 restriction. Curr Biol 2005; 15:73-8.
111. Ylinen L, Keckesova Z, Webb B, Gifford R, Smith T, Towers G. Isolation of an active Lv1 gene from cattle indicates that tripartite motif protein-mediated innate immunity to retroviral infection is widespread among mammals. J Virol 2006;80:7332-8.