Published in final edited form as:

Hum Gene Ther. 2005 October ; 16(10): 1125–1132. doi:10.1089/hum.2005.16.1125.

Control of Viral Infectivity by Tripartite Motif Proteins

GREG J. TOWERS

Wohl Virion Centre, Infection and Immunity, University College London, London W1T 4JF, UK.

Abstract

It is of great interest to understand the molecular details of the pathways that constitute species barriers to viral infection. The tripartite motif protein TRIM5 α has emerged as an important mediator of species-specific retroviral replication and innate immunity. This review considers the role of TRIM5 α as an antiviral protein in mammals. The methods used to identify species-specific restriction to retroviral infection, and the identification of TRIM5 α itself, are outlined. TRIM5 α mediates an early postentry block to sensitive retroviral infection, usually before viral DNA synthesis. Results from mutational analysis of TRIM5 α and their contribution to a mechanistic model for TRIM5 α antiviral activity are discussed. The antiviral role of other TRIM proteins is considered, as is the role of TRIM5 α cytoplasmic bodies.

INTRODUCTION

Retroviral infections are responsible for significant disease in mammals, causing a variety of pathologies including immune deficiency, malignancies, and neurological and immunological symptoms. They are not strictly species specific and on rare occasions are able to jump from one species to another, a process known as zoonosis. If a zoonotic virus is able to replicate in its new host and infect further individuals it can cause widespread disease. Human immunodeficiency viruses HIV-1 and HIV-2 have transferred into humans from chimpanzees and sooty mangabeys respectively (Gao *et al.*, 1992, 1999). Neither virus causes disease in its simian host but both cause acquired immunodeficiency syndrome (AIDS) in humans. Further examples of zoonotic viruses are the coronavirus from Chinese civet cats, which causes severe acute respiratory syndrome (Li *et al.*, 2005), as well as avian influenzaviruses that periodically cause influenza pandemics (Li *et al.*, 2004). It is of great interest to understand how mammalian species barriers ensure that zoonotic viral transfer is rare and to identify the molecular mechanisms involved. It is also important to understand how viruses bypass species barriers as this information might aid the design of novel antiviral therapeutics as well as help predict which viruses pose the greatest future threat to human health.

Phylogenetic analysis of HIV-1 sequences from infected individuals has revealed three HIV-1 groups termed M (main/major), O (outlier/other), and N (non-M, non-O/new), each resulting from an independent zoonosis into humans (Sharp *et al.*, 2001). Similarly, HIV-2 sequences reveal eight independent zoonotic transfers of SIVsm (simian immunodeficiency virus from sooty mangabeys) from sooty mangabeys into humans (Apetrei *et al.*, 2005). Remarkably, of these 11 transfers, only that which led to the HIV-1 M group of sequences is responsible for the AIDS pandemic, currently numbering about 40 million infected individuals and more than 20 million casualties (Marais, 2004). HIV-1 groups O and N are

epidemiologically unsuccessful and have led to a relatively small number of cases, mostly in West Africa. HIV-2 sequence types C to H each comprise a single sequence and almost certainly represent dead-end infections that have not been able to spread in humans. It is likely that many more viruses have transferred into humans, been unable to spread, and remain undetected. These observations illustrate the power of species barriers that protect us from viral infections from our simian cousins as well as from more distantly related animals.

Work on host factors that control viral permissivity has focused attention on intracellular antiviral proteins that have been referred to as restriction factors (Goff, 2004a,b). These proteins are part of an innate or intrinsic immune system that actively blocks viral infection in a species-specific way (Bieniasz, 2004). Certain members of a family of deaminase enzymes, exemplified by human APOBEC3G (*apolipoprotein B* mRNA-editing enzyme catalytic polypeptide-like 3G), are able to deaminate viral nucleic acids, leading to hypermutation and abortive infection (Sheehy *et al.*, 2002). The sole function of primate lentiviral Vif proteins appears to be to recruit APOBECs to the proteasome and allow successful viral replication (Harris *et al.*, 2003; Mangeat *et al.*, 2003; Sheehy *et al.*, 2003). A second family of antiviral proteins is defined by a tripartite motif and exemplified by the tripartite motif protein TRIM5 α (Stremlau *et al.*, 2004). This review focuses on identification of TRIM5 α as an important antiviral protein in primates, on its mechanism of action, and on whether TRIM5 α is unique within the TRIM family as having antiviral activity.

ASSAY FOR VIRAL INFECTIVITY

The science of gene therapy has driven the design of increasingly sophisticated viral vector systems for the delivery of therapeutic genes to patient cells (Sinn *et al.*, 2005). Retroviral vectors, derived from a range of retroviruses, have been useful for the study of the control of viral permissivity (Fig. 1). The vectors can be coated or “pseudotyped” with the envelope protein from vesicular stomatitis virus, thereby obviating specific envelope/receptor requirements and allowing infection of almost all cell lines tested. They can be engineered to encode green fluorescent protein and retroviral infection can be measured by fluorescence-activated cell sorting (FACS). Cell lines from a range of species have been tested for permissivity and the results are summarized in Table 1 (Hoffman *et al.*, 1999; Towers *et al.*, 2000; Besnier *et al.*, 2002; Cowan *et al.*, 2002; Ikeda *et al.*, 2002; Munk *et al.*, 2002; Hatzioannou *et al.*, 2003; Ylinen *et al.*, 2005). In each case BALB/c (B)-tropic murine leukemia virus tagged with green fluorescent protein (MLV-B GFP) is infectious, allowing the demonstration that the cells are specifically able to block infection of the restriction-sensitive viruses.

SATURATION OR ABROGATION ASSAYS REVEAL DOMINANT RESTRICTION FACTORS IN A RANGE OF SPECIES

Cells might be nonpermissive to retroviral infection for one of two reasons. It might be that the cells lack a factor that the restricted virus requires for the completion of its life cycle, or it might be that the cells express a factor that specifically blocks the incoming restricted virus. To differentiate between these two possibilities, saturation or “abrogation” assays have been used (Boone *et al.*, 1990; Besnier *et al.*, 2002; Cowan *et al.*, 2002; Towers *et al.*, 2002; Hatzioannou *et al.*, 2003). In these experiments virus-like particles (VLPs; in general, retroviral vector encoding puromycin resistance, rather than GFP) were titrated onto nonpermissive cell lines in the presence of a fixed low dose of virus encoding GFP. If the GFP-encoding virus became more infectious in the presence of the VLPs, in a VLP concentration-dependent way, then a dominant antiviral factor is present that the VLPs titrate, making the cells more permissive to the GFP-encoding virus. These experiments

revealed that in most cases, low permissivity to retroviral infection could indeed be titrated by VLPs, indicating that antiviral factors are common in mammalian cell lines. Nonsaturable examples include HIV-1 in rabbit cells and SIVmac (simian immunodeficiency virus from rhesus macaques) in squirrel monkey cells. In squirrel monkey cells, squirrel monkey TRIM5 α is responsible for SIVmac restriction but the rabbit restriction factor has not yet been identified (Besnier *et al.*, 2002; Ylinen *et al.*, 2005). Where saturation occurs, only VLPs that are sensitive to the particular restriction factor are able to saturate restriction, suggesting that a sensitive virus can interact with the antiviral factor, whereas an insensitive virus cannot. The antiviral factors have been termed restriction factors; the human factor is named Ref1 and the simian factor is named Lv1 (Towers *et al.*, 2000; Cowan *et al.*, 2002). Saturation assays have also been used effectively to demonstrate that in species in which infection by a number of unrelated viruses is blocked, such as humans and African green monkeys, saturation with VLPs from any of the sensitive viruses can render the cells permissive to restricted virus (Hatzioannou *et al.*, 2003). This observation strongly suggested that there was a single factor, now known to be TRIM5 α , that was able to recognize broadly unrelated viruses. The blocks to infection are realized early after infection, usually resulting in a strong inhibition of viral DNA synthesis. However, squirrel monkey TRIM5 α can block SIVmac infection without blocking DNA synthesis (Ylinen *et al.*, 2005), and this is discussed further below.

TRIM5 α IS A RESTRICTION FACTOR IN PRIMATES

The identification of TRIM5 α as a potent restriction factor, active against HIV-1 in rhesus macaques (Stremlau *et al.*, 2004), revolutionized the study of the control of retroviral permissivity. On the basis that rhesus macaque cells were poorly permissive to infection by HIV-1, Stremlau screened a rhesus macaque cDNA library for cDNAs that could block HIV-1 GFP infectivity. Cells expressing the cDNA library were repeatedly infected with HIV-1 GFP and the infected cells discarded. The resultant HIV-1 resistant clones were found to encode rhesus TRIM5 α . When TRIM5 α was expressed in human HeLa cells they became nonpermissive to HIV-1 GFP infection but permissivity to Moloney MLV was unaffected. On publication of this work a number of groups immediately set out to test whether human and simian TRIM5 α alleles could explain the examples of poor species-specific retroviral infectivity summarized in Table 1. It was quickly shown that the human TRIM5 α gene was indeed responsible for the low titer of MLV-N in human cells and for the low titer of HIV-1, SIVmac, equine infectious anemia virus (EIAV), and MLV-N in cells from African green monkeys (Hatzioannou *et al.*, 2004a; Keckesova *et al.*, 2004; Perron *et al.*, 2004; Yap *et al.*, 2004). Restricted virus infectivity was rescued by specifically reducing TRIM5 α expression, using small interfering RNA (siRNA). Furthermore, expression of TRIM5 α in permissive cells such as feline CRFK cells or murine MDTF cells rendered them able to restrict viral infection with the expected specificities. More recently, TRIM5 α has been shown to explain restriction of SIVmac in cells from squirrel monkeys and of HIV-2 in cells from rhesus macaques (Song *et al.*, 2005; Ylinen *et al.*, 2005).

Tripartite motif proteins, also known as RBCC proteins, are named for a series of three motifs with a characteristic order and spacing (Reymond *et al.*, 2001). They comprise a RING (really interesting new gene) domain encoding two atypical zinc fingers, either one or two B-box 2 domains, and a coiled coil domain. Some TRIM proteins, including TRIM5 α , additionally contain a SPRY domain, also found in members of the immunoglobulin superfamily. The B-box, coiled coil, and SPRY domains are likely to function in protein-protein interactions. Only the TRIM5 α splice variant, the longest and only one containing a SPRY domain, is able to block retroviral infection, indicating the importance of the SPRY domain for restriction (Stremlau *et al.*, 2004).

MUTATIONAL ANALYSIS OF TRIM5 α

Sequence comparison between *TRIM5 α* alleles from different primates suggested that variable regions in the SPRY domain might be responsible for their differential species-specific antiviral properties. A number of groups performed mutational analyses on *TRIM5 α* , swapping sections of the SPRY domains between human and simian *TRIM5 α* genes and testing the resultant fusions for antiviral activity (Nakayama *et al.*, 2005; Perez-Caballero *et al.*, 2005; Sawyer *et al.*, 2005; Stremlau *et al.*, 2005; Yap *et al.*, 2005). Together these studies concluded that a variable section of the SPRY domain, between approximately residues 320 and 345, was responsible for much of the species-specific restriction activity of TRIM5, although residues in the coiled coil were also important, particularly for restriction of MLV (Yap *et al.*, 2005). Notably, changing residue 332 in the SPRY domain of human TRIM5 to the corresponding residue in rhesus and African green monkey TRIM5, R332P, enabled the human protein to restrict HIV-1, although not as strongly as the wild-type rhesus protein (Stremlau *et al.*, 2005; Yap *et al.*, 2005). Interestingly, this mutant was also able to strongly restrict SIVmac, a property not shared by either of the wild-type *TRIM5 α* proteins (Stremlau *et al.*, 2005). This result is reminiscent of the observation that combining the antiviral determinants of the murine restriction factor Fv1 can result in an Fv1 mutant with novel anti-MLV specificities (Bock *et al.*, 2000). The corresponding *TRIM5 α* proline-to-arginine mutation in the rhesus protein also strengthened its ability to restrict HIV-1, further confirming the importance of this position in antiviral specificity (Yap *et al.*, 2005). In certain cases it is also possible to fuse the SPRY domain from one TRIM to the RBCC domain of a second TRIM and confer the antiviral specificity of the first to the fusion protein (Yap *et al.*, 2005). Together these observations indicate that the SPRY domain encodes the determinants of antiviral specificity.

TRIM5 is multiply spliced, resulting in at least three isoforms, each increasingly shorter from the C terminus. The TRIM5 γ isoform, which lacks the C-terminal SPRY domain, has dominant negative activity against the antiviral activity of TRIM5 α (Stremlau *et al.*, 2004). This activity is mediated through the coiled coil domain, which is necessary and sufficient for dominant negative activity (Perez-Caballero *et al.*, 2005). Furthermore, dominant negative activity has been shown to correlate with the ability to multimerize, in a yeast two-hybrid assay, and it therefore seems likely that molecules with a coiled coil, but without a SPRY domain, can heterodimerize with TRIM5 α to create a complex with a single SPRY domain that is unable to restrict infection (Perez-Caballero *et al.*, 2005). This hypothesis holds that multivalent interactions are required between the TRIM5 α complex and the virus for effective restriction. Indeed, one study has shown that capsid protein from restriction-sensitive MLV-N, but not from restriction-insensitive MLV-B, can be copurified with TRIM5 α in a SPRY domain-dependent way (Sebastian and Luban, 2005). This interaction is apparent only when using detergent-stripped virions, suggesting that the interaction occurs between TRIM5 α multimers and capsid multimers in the context of the intact virion core. Both lentiviral (HIV-1) and gammaretroviral (MLV) cores are made up of similar arrays of hexameric rings of capsid monomers (Li *et al.*, 2000; Mortuza *et al.*, 2004), supporting a similar mechanism for restriction of viruses unrelated at the sequence level. Whether multiple interactions are required because the TRIM5 α is required to bind and cross-link multiple hexamers or whether this requirement simply reflects the increased affinity of multiple binding sites remains unclear.

The TRIM5 RING domain has been shown to act as a ligase for autoubiquitination *in vitro* in the context of the TRIM5 δ splice variant (Xu *et al.*, 2003). This observation suggests that restriction might involve ubiquitination of the incoming capsid. Ubiquitination can have complex consequences and may not simply lead to recruitment to the proteasome (Weissman, 2001). Ubiquitination of incoming core could lead to mislocalization, alteration

of conformation, or simply perturbation of capsid rearrangement. However, whether the TRIM5 α RING domain plays a role in restriction remains unclear. TRIM5 α with the critical RING domain zinc-coordinating cysteine residues mutated clearly has reduced antiviral activity, although this mutant retains significant antiviral activity when overexpressed (Stremlau *et al.*, 2004; Javanbakht *et al.*, 2005). The role of the RING domain therefore remains uncertain. However, it is required for maximal restriction and we must be careful in assuming that it is redundant, because TRIM5 α RING mutants retain some anti-viral activity when overexpressed. It is also possible, however, that the TRIM5 α RING domain is required for a function unrelated to TRIM5 α antiviral activity.

VIRAL DETERMINANT FOR TRIM5 α RESTRICTION IS IN THE CAPSID

That the viral determinant for TRIM5 α restriction is in the capsid was originally shown by differential restriction of MLV-N, which is restricted in a range of species, and the closely related MLV-B, which is not (Towers *et al.*, 2000; Besnier *et al.*, 2003). This difference in tropism depends on a single amino acid in the MLV capsid: arginine in MLV-N promotes sensitivity to TRIM5 α restriction, whereas glutamate renders MLV-B insensitive to restriction. TRIM5 α sensitivity determinants are also found in the HIV-1 capsid and mutations to residues on the capsid surface alter HIV-1 titer in a species-specific way (Kootstra *et al.*, 2003; Hatzioannou *et al.*, 2004b; Ikeda *et al.*, 2004). More recent work considering HIV-2 and SIVmac has shown that changes in the external loop of the SIVmac capsid, homologous to the HIV-1 cyclophilin-binding site, can render SIVmac insensitive to TRIM5 α -mediated restriction in squirrel monkey cells, while retaining its insensitivity to human and rhesus TRIM5 α s (Ylinen *et al.*, 2005).

MODEL FOR RESTRICTION OF RETROVIRAL INFECTIVITY

The experimental data can be used to propose a mechanism for TRIM5 α -mediated restriction. Mapping the viral and TRIM5 α sensitivity determinants to the capsid (CA) and SPRY domains, respectively, suggests that the SPRY domain interacts directly with the incoming viral CA. What happens after TRIM5 α -CA interactions is unclear. It is reasonable to assume that, simply by interacting with multiple CA molecules in the incoming core, TRIM5 α can perturb the continuation of the infectious cycle. MLV and HIV-1 are known to have different uncoating requirements, with HIV-1 shedding most of its CA early after infection and MLV holding on to its CA during DNA synthesis (Fassati and Goff, 1999, 2001). However, it is likely that both viruses need to rearrange their viral core, possibly after specific interaction with host factors, before reverse transcription can efficiently proceed. In support of a role for CA in reverse transcription there are a number of viral CA mutants that form apparently normal particles but are unable to reverse transcribe (Craven *et al.*, 1995; Alin and Goff, 1996; Cairns and Craven, 2001). One explanation for this phenotype is that the mutant capsids cannot efficiently perform the rearrangements required for reverse transcription.

Interaction with TRIM5 α , either directly or indirectly, leads to a block to reverse transcription in most cases. However, squirrel monkey TRIM5 α , active against SIVmac, does not block SIVmac DNA synthesis (Ylinen *et al.*, 2005). Although this observation might suggest a later interaction with the virus, after DNA synthesis, it seems likely that squirrel monkey TRIM5 α interacts with virus early after infection but that the perturbation of capsid activity is subtle enough to disrupt viral infectivity, without significantly affecting reverse transcription.

ROLE OF CYTOPLASMIC BODIES REMAINS UNCLEAR

Certain TRIM proteins including TRIM5 α have been reported to exist in discrete areas of the cytoplasm, known as cytoplasmic bodies, when exogenously expressed (Reymond *et al.*, 2001). Whether endogenous TRIM5 α forms cytoplasmic bodies is unclear, but it appears that bodies are not required for TRIM5 α antiviral activity (Perez-Caballero *et al.*, 2005). The issue is complicated by the fact that many proteins, including TRIM5 α , form bodies called aggresomes when they are over-expressed (Kopito, 2000). Differentiating between true cytoplasmic bodies and aggresomes is challenging as they may be functionally related. Aggresomes are not simply spontaneously forming protein aggregates, as illustrated by their involvement in the regulation of the cellular enzyme inducible nitric oxide synthetase (Kolodziejaska *et al.*, 2005). It will be interesting to look for endogenous TRIM5 α cytoplasmic bodies, using TRIM5 α -specific antibodies, and to determine the effects of manipulations that affect restriction such as viral infection and drugs such as cyclosporin A.

ROLE FOR OTHER TRIM PROTEINS IN VIRAL PERMISSIVITY

The human genome encodes about 60 TRIM genes and it is obviously of great interest to consider whether there are more TRIM proteins with antiviral activity. There is much circumstantial evidence supporting an antiviral role for PML, the most well-studied TRIM protein, otherwise known as TRIM19. PML is found fused to retinoic acid receptor (RAR) by chromosome translocations in a series of malignancies including *promyelocytic leukemia*, after which it is named. The PML–RAR fusion deregulates both PML and retinoic acid receptor function and causes the malignant phenotype (Zelent *et al.*, 2001). In the nucleus PML exists in bodies called PML or ND10 bodies (Negorev and Maul, 2001). The cellular functions of the bodies and of PML are not completely solved but PML clearly has roles in the control of apoptosis, transcription, DNA repair, chromatin metabolism, and cell signaling (Borden, 2002; Lin *et al.*, 2004). Many studies have shown that replicating viral DNA tends to associate with PML bodies and that viral immediate-early gene transcription takes place there (Everett, 2001). Furthermore, the bodies are often disrupted by viral infection; usually the association between virus and the bodies is best seen with viral mutants that do not affect them. The degradation of PML by herpes simplex virus type 1 (HSV-1) infection has been well characterized. HSV-1-encoded E3 ubiquitin ligase ICP0 catalyzes PML ubiquitinylation and its recruitment to the proteasome (Chelbi-Alix and de Thé, 1999; Maul *et al.*, 2003). These observations imply that PML has antiviral activity and that certain viruses need to degrade or move PML in order to complete their life cycle. Further circumstantial evidence of an antiviral role for PML is provided in a number of studies including reduced permissivity to vesicular stomatitis virus and influenza A of hamster cells overexpressing PML. Moreover, cells derived from PML knockout mice have an impaired antiviral interferon response to HSV-1 infection, suggesting that PML is an essential component of the interferon response in these cells (Chee *et al.*, 2003). However, absolute proof of an antiviral role for PML remains elusive and the issue is controversial. An elegant study has shown that PML bodies form in association with transcriptionally active HSV-1 nucleoprotein complexes shortly after their arrival in the nucleus of an infected cell (Everett and Murray, 2005). The HSV-1 protein ICP0 then disrupts PML bodies associated with HSV-1 complexes as well as unassociated PML bodies. This work shows that the cell can deposit PML body proteins at specific sites in response to viral replication. Whether this is a defensive act remains unclear. PML has also been suggested to have anti-HIV-1 activity in human cells, although this observation is controversial (Turelli *et al.*, 2001).

As yet there is no strong evidence of further *TRIM* genes having antiviral roles. *TRIM1* genes from human, African green monkey, and owl monkey cells have been reported to

weakly restrict MLV, but only by a few fold, and it remains to be seen whether such restriction might be strong enough to be protective (Yap *et al.*, 2004).

ROLE OF CYCLOPHILIN A IN RESTRICTION

An interesting twist in the TRIM5 tale was provided by identification of the factor responsible for the low titer of HIV-1 in owl monkey cells (Nisole *et al.*, 2004; Sayah *et al.*, 2004). Owl monkeys are New World monkeys and an exception to the general rule that New World monkey cells are permissive to HIV-1 infection (Hofmann *et al.*, 1999). In cells from owl monkeys HIV-1 can be rendered insensitive to restriction by preventing interactions between the HIV-1 capsid protein and the immunophilin molecule cyclophilin A (CypA) (Towers *et al.*, 2003). Immunophilins comprise three families of abundant enzymes able to isomerize proline residues. Examples of peptidyl prolyl isomerase function suggest a role in the control of protein function. Isomerization of prolines by Pin1 has been associated with modulation of phosphorylation status, protein–protein interactions, subcellular localization, and protein stability (Ryo *et al.*, 2003). HIV-1 and CypA have a long history. CypA was identified in a yeast two-hybrid assay as a binding partner for HIV-1 Gag (Luban *et al.*, 1993). It was later shown that this interaction led to CypA being incorporated into the HIV-1 virions and mutations that blocked the interaction, as well as drugs that competed the CypA away from HIV-1, lowered HIV-1 infectivity in human cells (Franke *et al.*, 1994a,b; Thali *et al.*, 1994). It was assumed that HIV-1 capsids required CypA in order to fold properly or to properly uncoat and efficiently reverse transcribe. Prior to the identification of TRIM5 α as encoding Ref1/Lv1 a role for restriction was hypothesized for the anti-HIV-1 effects of cyclosporin A (CSA) in human cells (Towers *et al.*, 2003). However, the role of human TRIM5 α , if any, in the effects of CSA remains unclear.

The most important CA–CypA interactions take place after the virus has entered the target cells, compared with those that incorporate CypA into HIV-1 particles (Kootstra *et al.*, 2003; Towers *et al.*, 2003; Sokolskaja *et al.*, 2004; Hatzioannou *et al.*, 2005). Experiments in owl monkeys showed that the effects of CypA were also important in monkeys, but in this case CypA–CA interactions were inhibitory. The same mutations or drugs that decreased titer in human cells completely rescued restricted infectivity in owl monkey cells to high titer (Towers *et al.*, 2003). These observations were followed by those of Sayah *et al.*, who were intrigued by the observation that whereas knocking down CypA expression with siRNA rescued HIV-1 infectivity in owl monkey cells, replacing the CypA with an siRNA-insensitive CypA did not return the cells to their nonpermissive state (Sayah *et al.*, 2004). This observation implied that there was a second CypA-related protein in owl monkey cells that was essential for restriction of HIV-1. Sayah and coworkers identified a fusion protein between TRIM5 and CypA, expressed uniquely in owl monkeys, that explained the sensitivity of HIV-1 to manipulation of CA–CypA interactions. It appears that a CypA pseudogene had inserted into exon 7 of the *TRIM5* gene by retrotransposition to produce the TRIM–Cyp allele. This finding was quickly followed by Nisole *et al.*, who found the same TRIM–Cyp fusion in owl monkey cells and demonstrated that TRIM–Cyp binds the HIV-1 capsid through its CypA domain *in vitro* (Nisole *et al.*, 2004). Owl monkeys appear to be unique in encoding a TRIM–Cyp fusion. The CypA pseudogene replaces the SPRY domain and presumably has the same function in bringing the RBCC domain into contact with the incoming HIV-1 core. The presence of the CypA domain therefore explains the absolute sensitivity of HIV-1 restriction in owl monkey cells to CSA and mutations such as HIV-1 CA G89V, which prevent CypA, and therefore TRIM–Cyp, from interacting with the HIV-1 capsid. It is intriguing, and as yet unexplained, that restricted HIV-1 infectivity is also rescued in Old World monkey cells such as African green monkey CV1 cells and rhesus FRhK4 cells by CSA treatment (Ikeda *et al.*, 2004). The reason for this TRIM–Cyp-

independent CSA sensitivity is likely to be mechanistically important and warrants further investigation.

FUTURE FOR POSTENTRY RESTRICTION RESEARCH

There remain a number of examples of poor permissivity to retroviral infection for which the factor(s) responsible have not yet been identified. The Lv2 phenotype (Schmitz *et al.*, 2004) and the restriction of certain lentiviruses in HeLa cells (Sokolskja *et al.*, 2004; Hatzioannou *et al.*, 2005) are clearly TRIM5 independent. Also, rodent cells (Hatzioannou *et al.*, 2004c), rabbit cells (Hofmann *et al.*, 1999; Besnier *et al.*, 2002), and bat, cow, and pig cells (Towers *et al.*, 2000) restrict retroviral infection, possibly through TRIM5. MLV-N restriction in hamster and dog cells (Towers *et al.*, 2000) has not survived closer examination (G.J. Towers, unpublished observations). The factors responsible for these restriction phenotypes are likely to be identified soon and they may swell the ranks of antiviral TRIM proteins.

The fact that HIV-2 can be restricted by human TRIM5 when overexpressed suggests that it may play a role in controlling HIV-2 infection in humans (Ylinen *et al.*, 2005). Further analysis of these observations with primary cells and virus is likely to be informative. It will be interesting to characterize TRIM5 polymorphisms in the human population and to determine whether these could have any impact on HIV-1 or HIV-2 replication. The role of cytoplasmic bodies, if any, in restriction is an interesting question, as is whether TRIM5 has functions unrelated to its antiviral properties.

As well as being a cause of disease, retroviruses have shown great promise in the delivery of therapeutic genes in gene therapy protocols. A significant hurdle in successful gene therapy is the efficient delivery of the therapeutic gene to the correct target cells. Often the most important target cells, for example, stem cells, have low permissivity to retroviral infection. A better understanding of the host–virus interactions that determine permissivity will enable the design of retroviral vectors with higher infectivities in relevant cell types. Furthermore, understanding the determinants of species-specific retroviral replication will enable us to improve animal models for AIDS. Current models comprise rhesus macaques infected with simian immunodeficiency virus (SIVmac) and its derivatives as HIV-1 is strongly restricted by both rhesus TRIM5 α and APOBEC3G. The current models have been successful in understanding the immunology of AIDS (Mattapallil *et al.*, 2005), but their use in testing vaccines and anti-HIV therapeutics is limited. Altering HIV-1 so that it can replicate and cause disease in rhesus macaques would be expected to significantly improve the AIDS animal model. The study of host factors controlling viral permissivity is therefore likely to provide information that is both interesting and useful to a broad audience.

Acknowledgments

Research in the Towers laboratory is supported by the Well-come Trust, the University College London Graduate School, and the Yule and Charlotte Bogue Fellowship Scheme.

REFERENCES

- ALIN K, GOFF SP. Amino acid substitutions in the CA protein of Moloney murine leukemia virus that block early events in infections. *Virology*. 1996; 222:339–351. [PubMed: 8806518]
- APETREI C, KAUR A, LERCHE NW, METZGER M, PANDREA I, HARDCASTLE J, FALKENSTEIN S, BOHM R, KOEHLER J, TRAINA-DORGE V, WILLIAMS T, STAPRANS S, PLAUCHE G, VEAZEY RS, MCCLURE H, LACKNER AA, GORMUS B, ROBERTSON DL, MARX PA. Molecular epidemiology of simian immunodeficiency virus SIVsm in U.S. primate

- centers unravels the origin of SIVmac and SIVstm. *J. Virol.* 2005; 79:8991–9005. [PubMed: 15994793]
- BESNIER C, TAKEUCHI Y, TOWERS G. Restriction of lentivirus in monkeys. *Proc. Natl. Acad. Sci. U.S.A.* 2002; 99:11920–11925. [PubMed: 12154231]
- BESNIER C, YLINEN L, STRANGE B, LISTER A, TAKEUCHI Y, GOFF SP, TOWERS GJ. Characterization of murine leukemia virus restriction in mammals. *J. Virol.* 2003; 77:13403–13406. [PubMed: 14645596]
- BIENIASZ PD. Intrinsic immunity: A front-line defense against viral attack. *Nat. Immunol.* 2004; 5:1109–1115. [PubMed: 15496950]
- BOCK M, BISHOP K, TOWERS G, STOYE JP. Use of a transient assay for studying the genetic determinants of Fv1 restriction. *J. Virol.* 2000; 74:7422–7430. [PubMed: 10906195]
- BOONE LR, INNES CL, HEITMAN CK. Abrogation of Fv-1 restriction by genome-deficient virions produced by a retrovirus packaging cell line. *J. Virol.* 1990; 64:3376–3381. [PubMed: 1693703]
- BORDEN KL. Pondering the promyelocytic leukemia protein (PML) puzzle: Possible functions for PML nuclear bodies. *Mol. Cell. Biol.* 2002; 22:5259–5269. [PubMed: 12101223]
- CAIRNS TM, CRAVEN RC. Viral DNA synthesis defects in assembly-competent Rous sarcoma virus CA mutants. *J. Virol.* 2001; 75:242–250. [PubMed: 11119594]
- CHEE AV, LOPEZ P, PANDOLFI PP, ROIZMAN B. Promyelocytic leukemia protein mediates interferon-based anti-herpes simplex virus 1 effects. *J. Virol.* 2003; 77:7101–7105. [PubMed: 12768029]
- CHELBI-ALIX MK, DE THE H. Herpes virus induced proteasome-dependent degradation of the nuclear bodies-associated PML and Sp100 proteins. *Oncogene.* 1999; 18:935–941. [PubMed: 10023669]
- COWAN S, HATZIOANNOU T, CUNNINGHAM T, MUESING MA, GOTTLINGER HG, BIENIASZ PD. Cellular inhibitors with Fv1-like activity restrict human and simian immunodeficiency virus tropism. *Proc. Natl. Acad. Sci. U.S.A.* 2002; 99:11914–11919. [PubMed: 12154227]
- CRAVEN R, LEURE-DUPREE AE, WELDON RA, WILLS JW. Genetic analysis of the major homology region of the Rous sarcoma virus Gag protein. *J. Virol.* 1995; 69:4213–4227. [PubMed: 7769681]
- EVERETT RD. DNA viruses and viral proteins that interact with PML nuclear bodies. *Oncogene.* 2001; 20:7266–7273. [PubMed: 11704855]
- EVERETT RD, MURRAY J. ND10 components relocate to sites associated with herpes simplex virus type 1 nucleoprotein complexes during virus infection. *J. Virol.* 2005; 79:5078–5089. [PubMed: 15795293]
- FASSATI A, GOFF SP. Characterization of intracellular reverse transcription complexes of Moloney murine leukemia virus. *J. Virol.* 1999; 73:8919–8925. [PubMed: 10515996]
- FASSATI A, GOFF SP. Characterization of intracellular reverse transcription complexes of human immunodeficiency virus type 1. *J. Virol.* 2001; 75:3626–3635. [PubMed: 11264352]
- FRANKE EK, YUAN HE, LUBAN J. Specific incorporation of cyclophilin A into HIV-1 virions. *Nature.* 1994a; 372:359–362. [PubMed: 7969494]
- FRANKE EK, YUAN HE, BOSSOLT KL, GOFF SP, LUBAN J. Specificity and sequence requirements for interactions between various retroviral Gag proteins. *J. Virol.* 1994b; 68:5300–5305. [PubMed: 8035530]
- GAO F, YUE L, WHITE AT, PAPPAS PG, BARCHUE J, HANSON AP, GREENE BM, SHARP PM, SHAW GM, HAHN BH. Human infection by genetically diverse SIVSM-related HIV-2 in West Africa. *Nature.* 1992; 358:495–499. [PubMed: 1641038]
- GAO F, BAILES E, ROBERTSON DL, CHEN Y, RODENBURG CM, MICHAEL SF, CUMMINS LB, ARTHUR LO, PEETERS M, SHAW GM, SHARP PM, HAHN BH. Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes*. *Nature.* 1999; 397:436–441. [PubMed: 9989410]
- GOFF SP. Retrovirus restriction factors. *Mol. Cell.* 2004a; 16:849–859. [PubMed: 15610729]
- GOFF SP. Genetic control of retrovirus susceptibility in mammalian cells. *Annu. Rev. Genet.* 2004b; 38:61–85. [PubMed: 15568972]

- HARRIS RS, BISHOP KN, SHEEHY AM, CRAIG HM, PETERSEN-MAHRT SK, WATT IN, NEUBERGER MS, MALIM MH. DNA deamination mediates innate immunity to retroviral infection. *Cell*. 2003; 113:803–809. [PubMed: 12809610]
- HATZIOANNOU T, COWAN S, GOFF SP, BIENIASZ PD, TOWERS GJ. Restriction of multiple divergent retroviruses by Lv1 and Ref1. *EMBO J*. 2003; 22:385–394. [PubMed: 12554640]
- HATZIOANNOU T, PEREZ-CABALLERO D, YANG A, COWAN S, BIENIASZ PD. Retrovirus resistance factors Ref1 and Lv1 are species-specific variants of TRIM5 α . *Proc. Natl. Acad. Sci. U.S.A.* 2004a; 101:10774–10779. [PubMed: 15249685]
- HATZIOANNOU T, COWAN S, VON SCHWEDLER UK, SUNDQUIST WI, BIENIASZ PD. Species-specific tropism determinants in the human immunodeficiency virus type 1 capsid. *J. Virol.* 2004b; 78:6005–6012. [PubMed: 15140998]
- HATZIOANNOU T, COWAN S, BIENIASZ PD. Capsid-dependent and independent postentry restriction of primate lentivirus tropism in rodent cells. *J. Virol.* 2004c; 78:1006–1011. [PubMed: 14694132]
- HATZIOANNOU T, PEREZ-CABALLERO D, COWAN S, BIENIASZ PD. Cyclophilin interactions with incoming human immunodeficiency virus type 1 capsids with opposing effects on infectivity in human cells. *J. Virol.* 2005; 79:176–183. [PubMed: 15596813]
- HOFMANN W, SCHUBERT D, LABONTE J, MUNSON L, GIBSON S, SCAMMELL J, FERRIGNO P, SODROSKI J. Species-specific, postentry barriers to primate immunodeficiency virus infection. *J. Virol.* 1999; 73:10020–10028. [PubMed: 10559316]
- IKEDA Y, COLLINS MK, RADCLIFFE PA, MITROPHANOUS KA, TAKEUCHI Y. Gene transduction efficiency in cells of different species by HIV and EIAV vectors. *Gene Ther.* 2002; 9:932–938. [PubMed: 12085241]
- IKEDA Y, YLINEN L, KAHAR-BADOR M, TOWERS GJ. The influence of Gag on HIV-1 species specific tropism. *J. Virol.* 2004; 78:11816–11822. [PubMed: 15479823]
- 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 TRIM5 α . *J. Biol. Chem.* 2005; 280:26933–26940. [PubMed: 15897199]
- KECKESOVA Z, YLINEN LM, TOWERS GJ. The human and African green monkey TRIM5 α genes encode Ref1 and Lv1 retroviral restriction factor activities. *Proc. Natl. Acad. Sci. U.S.A.* 2004; 101:10780–10785. [PubMed: 15249687]
- KOŁODZIEJSKA KE, BURNS AR, MOORE RH, STENOIEN DL, EISSA NT. Regulation of inducible nitric oxide synthase by aggresome formation. *Proc. Natl. Acad. Sci. U.S.A.* 2005; 102:4854–4859. [PubMed: 15781872]
- KOOTSTRA NA, MUNK C, TONNU N, LANDAU NR, VERMA IM. Abrogation of postentry restriction of HIV-1-based lentiviral vector transduction in simian cells. *Proc. Natl. Acad. Sci. U.S.A.* 2003; 100:1298–1303. [PubMed: 12547912]
- KOPITO RR. Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol.* 2000; 10:524–530. [PubMed: 11121744]
- LI KS, GUAN Y, WANG J, SMITH GJ, XU KM, DUAN L, RAHARDJO AP, PUTHAVATHANA P, BURANATHAI C, NGUYEN TD, ESTOEPANGESTIE AT, CHAISINGH A, AUEWARAKUL P, LONG HT, HANH NT, WEBBY RJ, POON LL, CHEN H, SHORTRIDGE KF, YUEN KY, WEBSTER RG, PEIRIS JS. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. *Nature*. 2004; 430:209–213. [PubMed: 15241415]
- LI S, HILL CP, SUNDQUIST WI, FINCH JT. Image reconstructions of helical assemblies of the HIV-1 CA protein. *Nature*. 2000; 407:409–413. [PubMed: 11014200]
- LI W, ZHANG C, SUI J, KUHN JH, MOORE MJ, LUO S, WONG SK, HUANG IC, XU K, VASILIEVA N, MURAKAMI A, HE Y, MARASCO WA, GUAN Y, CHOE H, FARZAN M. Receptor and viral determinants of SARS coronavirus adaptation to human ACE2. *EMBO J*. 2005; 24:1634–1643. [PubMed: 15791205]
- LIN HK, BERGMANN S, PANDOLFI PP. Cytoplasmic PML function in TGF- β signalling. *Nature*. 2004; 431:205–211. [PubMed: 15356634]

- LUBAN J, BOSSOLT KL, FRANKE EK, KALPANA GV, GOFF SP. Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. *Cell*. 1993; 73:1067–1078. [PubMed: 8513493]
- MANGEAT B, TURELLI P, CARON G, FRIEDLI M, PERRIN L, TRONO D. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature*. 2003; 424:99–103. [PubMed: 12808466]
- MARAIS, H.; STANECKI, K. AIDS Epidemic Update. Joint United Nations Programme on HIV/AIDS; Geneva, Switzerland: 2004. Available at URL http://www.unaids.org/wad2004/EPI_1204_pdf_en/Chapter0_intro_en.pdf (accessed August 2005)
- MATTAPALLIL JJ, DOUEK DC, HILL B, NISHIMURA Y, MARTIN M, ROEDERER M. Massive infection and loss of memory CD4⁺ T cells in multiple tissues during acute SIV infection. *Nature*. 2005; 434:1093–1097. [PubMed: 15793563]
- MAUL GG, GULDNER HH, SPIVACK JG. Modification of discrete nuclear domains induced by herpes simplex virus type 1 immediate early gene 1 product (ICP0). *J. Gen. Virol.* 1993; 74:2679–2690. [PubMed: 8277273]
- MORTUZA GB, HAIRE LF, STEVENS A, SMERDON SJ, STOYE JP, TAYLOR IA. High-resolution structure of a retroviral capsid hexameric amino-terminal domain. *Nature*. 2004; 431:481–485. [PubMed: 15386017]
- MUNK C, BRANDT SM, LUCERO G, LANDAU NR. A dominant block to HIV-1 replication at reverse transcription in simian cells. *Proc. Natl. Acad. Sci. U.S.A.* 2002; 99:13843–13848. [PubMed: 12368468]
- NAKAYAMA EE, MIYOSHI H, NAGAI Y, SHIODA T. A specific region of 37 amino acid residues in the SPRY (B30.2) domain of African green monkey TRIM5 α determines species-specific restriction of simian immunodeficiency virus SIVmac infection. *J. Virol.* 2005; 79:8870–8877. [PubMed: 15994780]
- NEGOREV D, MAUL GG. Cellular proteins localized at and interacting within ND10/PML nuclear bodies/PODs suggest functions of a nuclear depot. *Oncogene*. 2001; 20:7234–7242. [PubMed: 11704851]
- NISOLE S, LYNCH C, STOYE JP, YAP MW. A Trim5-cyclophilin A fusion protein found in owl monkey kidney cells can restrict HIV-1. *Proc. Natl. Acad. Sci. U.S.A.* 2004; 101:13324–13328. [PubMed: 15326303]
- PEREZ-CABALLERO D, HATZIOANNOU T, YANG A, COWAN S, BIENIASZ PD. Human tripartite motif 5 α domains responsible for retrovirus restriction activity and specificity. *J. Virol.* 2005; 79:8969–8978. [PubMed: 15994791]
- PERRON MJ, STREMLAU M, SONG B, ULM W, MULLIGAN RC, SODROSKI J. TRIM5 α mediates the postentry block to N-tropic murine leukemia viruses in human cells. *Proc. Natl. Acad. Sci. U.S.A.* 2004; 101:11827–11832. [PubMed: 15280539]
- REYMOND A, MERONI G, FANTOZZI A, MERLA G, CAIRO S, LUZI L, RIGANELLI D, ZANARIA E, MESSALI S, CAINARCA S, GUFFANTI A, MINUCCI S, PELICCI PG, BALLABIO A. The tripartite motif family identifies cell compartments. *EMBO J.* 2001; 20:2140–2151. [PubMed: 11331580]
- RYO A, SUIZU F, YOSHIDA Y, PERREM K, LIOU YC, WULF G, ROTTAPPEL R, YAMAOKA S, LU KP. Regulation of NF- κ B signaling by Pin1-dependent prolyl isomerization and ubiquitin-mediated proteolysis of p65/RelA. *Mol. Cell*. 2003; 12:1413–1426. [PubMed: 14690596]
- SAWYER SL, WU LI, EMERMAN M, MALIK HS. Positive selection of primate TRIM5 α identifies a critical species-specific retroviral restriction domain. *Proc. Natl. Acad. Sci. U.S.A.* 2005; 102:2832–2837. [PubMed: 15689398]
- SAYAH DM, SOKOLSKAJA E, BERTHOUX L, LUBAN J. Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. *Nature*. 2004; 430:569–573. [PubMed: 15243629]
- SCHMITZ C, MARCHANT D, NEIL SJ, AUBIN K, REUTER S, DITTMAR MT, MCKNIGHT A. Lv2, a novel postentry restriction, is mediated by both capsid and envelope. *J. Virol.* 2004; 78:2006–2016. [PubMed: 14747565]

- SEBASTIAN S, LUBAN J. TRIM5 α selectively binds a restriction-sensitive retroviral capsid. *Retrovirology*. 2005; 2:40. [PubMed: 15967037]
- SHARP PM, BAILES E, CHAUDHURI RR, RODENBURG CM, SANTIAGO MO, HAHN BH. The origins of acquired immune deficiency syndrome viruses: Where and when? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 2001; 356:867–876. [PubMed: 11405934]
- SHEEHY AM, GADDIS NC, CHOI JD, MALIM MH. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature*. 2002; 418:646–650. [PubMed: 12167863]
- SHEEHY AM, GADDIS NC, MALIM MH. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. *Nat. Med.* 2003; 9:1404–1407. [PubMed: 14528300]
- SINN PL, SAUTER SL, MCCRAY PB. Gene therapy progress and prospects: Development of improved lentiviral and retroviral vectors—design, biosafety, and production. *Gene Ther.* 2005; 12:1089–1098. [PubMed: 16003340]
- SOKOLSKAJA E, SAYAH DM, LUBAN J. Target cell cyclophilin A modulates human immunodeficiency virus type 1 infectivity. *J. Virol.* 2004; 78:12800–12808. [PubMed: 15542632]
- SONG B, JAVANBAKHT H, PERRON M, PARK DO H, STREMLAU M, SODROSKI J. Retrovirus restriction by TRIM5 α variants from Old World and New World primates. *J. Virol.* 2005; 79:3930–3937. [PubMed: 15767395]
- STREMLAU M, OWENS CM, PERRON MJ, KIESSLING M, AUTISSIER P, SODROSKI J. The cytoplasmic body component TRIM5 α restricts HIV-1 infection in Old World monkeys. *Nature*. 2004; 427:848–853. [PubMed: 14985764]
- STREMLAU M, PERRON MJ, WELIKALA S, SODROSKI J. Species-specific variation in the B30.2(SPRY) domain of TRIM5 α determines the potency of human immunodeficiency virus restriction. *J. Virol.* 2005; 79:3139–3145. [PubMed: 15709033]
- THALI M, BUKOVSKY A, KONDO E, ROSENWIRTH B, WALSH CT, SODROSKI J, GOTTLINGER HG. Functional association of cyclophilin A with HIV-1 virions. *Nature*. 1994; 372:363–365. [PubMed: 7969495]
- TOWERS G, BOCK M, MARTIN S, TAKEUCHI Y, STOYE JP, DANOS O. A conserved mechanism of retrovirus restriction in mammals. *Proc. Natl. Acad. Sci. U.S.A.* 2000; 97:12295–12299. [PubMed: 11027299]
- TOWERS G, COLLINS M, TAKEUCHI Y. Abrogation of Ref1 restriction in human cells. *J. Virol.* 2002; 76:2548–2550. [PubMed: 11836433]
- TOWERS GJ, HATZIOANNOU T, COWAN S, GOFF SP, LUBAN J, BIENIASZ PD. Cyclophilin A modulates the sensitivity of HIV-1 to host restriction factors. *Nat. Med.* 2003; 9:1138–1143. [PubMed: 12897779]
- TURELLI P, DOUCAS V, CRAIG E, MANGEAT B, KLAGES N, EVANS R, KALPANA G, TRONO D. Cytoplasmic recruitment of INI1 and PML on incoming HIV preintegration complexes: Interference with early steps of viral replication. *Mol. Cell.* 2001; 7:1245–1254. [PubMed: 11430827]
- WEISSMAN AM. Themes and variations on ubiquitinylation. *Nat. Rev. Mol. Cell. Biol.* 2001; 2:169–178. [PubMed: 11265246]
- XU L, YANG L, MOITRA PK, HASHIMOTO K, RALLABHANDI P, KAUL S, MERONI G, JENSEN JP, WEISSMAN AM, D'ARPA P. BTBD1 and BTBD2 colocalize to cytoplasmic bodies with the RBCC/tripartite motif protein, TRIM5 α . *Exp. Cell Res.* 2003; 288:84–93. [PubMed: 12878161]
- YAP MW, NISOLE S, LYNCH C, STOYE JP. Trim5 α protein restricts both HIV-1 and murine leukemia virus. *Proc. Natl. Acad. Sci. U.S.A.* 2004; 101:10786–10791. [PubMed: 15249690]
- YAP MW, NISOLE S, STOYE JP. A single amino acid change in the SPRY domain of human Trim5 α leads to HIV-1 restriction. *Curr. Biol.* 2005; 15:73–78. [PubMed: 15649369]
- YLINEN L, KECKESOVA Z, WILSON SJ, RANASINGHE S, TOWERS GJ. Differential restriction of HIV-2 and SIVmac by TRIM5 α alleles. *J. Virol.* 2005 in press.
- ZELENT A, GUIDEZ F, MELNICK A, WAXMAN S, LICHT JD. Translocations of the RAR α gene in acute promyelocytic leukemia. *Oncogene*. 2001; 20:7186–7203. [PubMed: 11704847]

FIG. 1.

An assay for retroviral infectivity. Plasmids encoding a *gag-pol* expression cassette, a VSV-G (envelope protein from vesicular stomatitis virus) expression cassette, and a retroviral vector encoding GFP are transfected into 293T cells. Forty-eight hours later the supernatant is collected, filtered, and serially diluted. These dilutions are used to infect cells plated in six-well plates in the presence of Polybrene (5 µg/ml). Forty-eight hours later the green cells are enumerated by FACS and the percentage infection is plotted against the viral input dose, measured in reverse transcriptase units or infectious units per milliliter as determined with an indicator cell line.

Table 1**Retroviral Restrictions**

Species	Restriction-sensitive viruses	Restriction saturable	TRIM5 α required
Human (Ref1)	MLV-N, EIAV	Yes	Yes
African green monkey (Lv1)	MLV-N, HIV-1, HIV-2, SIVmac, EIAV	Yes	Yes
Owl monkey	HIV-1	Yes	Yes (TRIM-Cyp)
Rhesus macaque	HIV-1, HIV-2, MLV-N, SIVagm	Yes	Yes
Squirrel monkey	SIVmac	No	Yes
Cynomolgus monkey	HIV-1	Untested	Yes
Rabbit	HIV-1, HIV-2	No	Untested
Bats, cows	MLV-N	Untested	Untested
Pigs	MLV-N	Untested	Untested
Chimpanzee ^a	MLV-N	Untested	Yes
Spider monkey ^a	HIV-1, SIVagm	Untested	Yes
Orangutan ^a	MLV-N	Untested	Yes
Tamarin ^a	SIVmac	Untested	Yes

Abbreviations: EIAV, equine infectious anemia virus; HIV-1 and HIV-2, human immunodeficiency virus types 1 and 2; N-MLV, N-tropic murine leukemia virus; SIVagm, simian immunodeficiency virus from African green monkeys; SIVmac, simian immunodeficiency virus from rhesus macaques; TRIM5 α , tripartite motif protein 5 α ; TRIM-Cyp, TRIM with cyclophilin A insertion.

^aRestriction specificities were determined in permissive cells overexpressing the TRIM5 α alleles specified. In these cases experiments have not been performed in cell lines from the species indicated.