Trafficking of some old world primate TRIM5α proteins through the nucleus

Felipe Diaz-Griffero1, Daniel E Gallo4, Thomas J Hope4 and Joseph Sodroski2,3*

Abstract

Background: TRIM5α and TRIMCyp are cytoplasmic proteins that bind incoming retroviral capsids and mediate early blocks to viral infection. TRIM5 proteins form cytoplasmic bodies, which are highly dynamic structures. So far, TRIM5 proteins have been found only in the cytoplasm of cells. Interestingly, other proteins from the TRIM family localize to the nucleus. Therefore, we tested the possibility that TRIM5 proteins traffic to the nucleus and the impact of this trafficking on retroviral restriction.

Results: Here we report that the TRIM5α proteins of two Old World primates, humans and rhesus monkeys, are transported into the nucleus and are shuttled back to the cytoplasm by a leptomycin B-sensitive mechanism. In leptomycin B-treated cells, these TRIM5α proteins formed nuclear bodies that also contained TRIM19 (PML). Deletion of the amino terminus, including the linker 1 (L1) region, resulted in TRIM5α proteins that accumulated in nuclear bodies. Leptomycin B treatment of TRIM5α-expressing target cells only minimally affected the restriction of retrovirus infection.

Conclusions: We discovered the ability of human and rhesus TRIM5α to shuttle into and out of the nucleus. This novel trafficking ability of TRIM5α proteins could be important for an as-yet-unknown function of TRIM5α.

Keywords: Restriction factor intracellular localization, retrovirus, leptomycin B

Background

Proteins of the tripartite motif (TRIM) family contain RING, B-Box and coiled-coil domains, and thus have been referred to as RBCC proteins [1]. Members of this family have been implicated in diverse processes such as cell proliferation, differentiation, development, oncogenesis and apoptosis [1,2]. TRIM proteins often self-associate and, when overexpressed, aggregate to form nuclear or cytoplasmic bodies [1].

TRIM5α is a cytoplasmic protein that is capable of restricting retrovirus infection in a species-dependent manner [3]. Variation among TRIM5α proteins in different primates accounts for the early, post-entry blocks to infection by particular retroviruses [3-7]. For example, TRIM5α proteins of Old World monkeys block human immunodeficiency virus (HIV-1) infection [3-5,7], whereas TRIM5α proteins of New World monkeys block infection by simian immunodeficiency virus (SIVmac) [8]. TRIM5α from humans (TRIM5αhu) is not as potent in restricting HIV-1 infection as Old World monkey TRIM5α, but TRIM5αhu potently restricts other retroviruses, e.g., N-tropic murine leukemia virus (N-MLV) and equine infectious anemia virus (EIAV) [3,4,6-8]. Owl monkeys, a New World monkey species, are unusual in not expressing a TRIM5α protein, but instead express TRIMCyp, in which the RBCC domains of TRIM5 are fused to a cyclophilin A moiety [9,10].

Variation in splicing of the TRIM5 primary transcript leads to the expression of TRIM5 isoforms, designated α, γ and δ [1]. The TRIM5α isoform contains, in addition to the RING, B-box 2 and coiled-coil domains, a carboxy-terminal B30.2(SPRY) domain. The B30.2 (SPRY) domain is essential for the antiretroviral activity of TRIM5α [3]. In some cases, the differences in the ability of TRIM5α proteins from various primate species to restrict particular retroviruses are determined by sequences in the B30.2(SPRY) domain [11-19]. The B30.2(SPRY) domain in TRIM5α and the cyclophilin A...
domain in TRIMCyp allow these restriction factors to bind specifically to particular retroviral capsids [9,20-24]. Additional sequences in the B-box 2 domain contribute to higher-order self-association of TRIM5α, which allows higher avidity for the retroviral capsid [25-27]. TRIM5α proteins aggregate on the incoming retroviral capsid [28]; and, by as-yet-uncertain mechanisms, decrease the stability of the capsid [23,27,29,30].

Some TRIM proteins localize in the nucleus of cells. One example is TRIM19 (promyelocytic leukemia (PML) protein), which is a major component of nuclear domain 10 (ND10) bodies [31-33]. TRIM19 has been shown to interfere with the replication of several DNA and RNA viruses [34-41]. Both TRIM19 and TRIM5α can inhibit herpes simplex virus replication [34,40,41], and RNA viruses [28]; and, by as-yet-uncertain mechanisms, decrease the stability of the capsid [23,27,29,30].

To examine whether TRIM5αrh localizes to the same ND10 bodies as TRIM19 after LMB treatment, LMB-treated human cells stably expressing TRIM5αrh were stained with antibodies directed against TRIM19 and the hemagglutinin (HA) epitope tag on TRIM5αrh. The nuclear TRIM5αrh-GFP were evident by 2 hours following the initiation of LMB treatment.

Nuclear TRIM5αhu and TRIM5αrh proteins localize to ND10 bodies with TRIM19

To examine whether TRIM5αrh localizes to the same ND10 bodies as TRIM19 after LMB treatment, LMB-treated human cells stably expressing TRIM5αrh were stained with antibodies directed against TRIM19 and the hemagglutinin (HA) epitope tag on TRIM5αrh. The nuclear TRIM5αrh-GFP resulted in a rapid accumulation of TRIM5αrh-GFP in the nucleus (Figure 2). Nuclear bodies containing TRIM5αrh-GFP were evident by 2 hours following the initiation of LMB treatment.

Localization of a TRIM5αrh-pyruvate kinase fusion protein

The diameter of the nuclear pore is approximately 0.9 nm, which allows globular proteins less than 60 kD to diffuse freely through the channel [50-52]. TRIM5α proteins (approximately 55 kD) are close to this diffusion limit. Moreover, TRIM5α forms a stable dimer [20,21]; however, we do not know if the majority of TRIM5α molecules that enter the nucleus are monomers or dimers. In addition, the molecular shape of TRIM5α is unknown. These uncertainties raised the possibility that TRIM5α is actively transported into the nucleus. To test this possibility, TRIM5αrh was fused to pyruvate kinase (PK), which is normally a cytoplasmic protein [53] and to the green fluorescent protein (GFP) to create the GFP-PK-TRIM5αrh chimeric protein. The GFP-PK-TRIM5αrh protein and a control GFP-PK protein were transiently expressed in HeLa cells (Figure 4). Localization of these proteins was examined in untreated and LMB-treated cells (Figure 4). After a two-hour treatment with 10 nM LMB, the GFP-PK-TRIM5αrh protein was detected in both the nucleus and the cytoplasm. By contrast, the GFP-PK protein was detected only in the cytoplasm of untreated and LMB-treated cells.
are consistent with the active transport of TRIM5α<sub>rh</sub> to the nucleus.

**Identification of TRIM5α<sub>rh</sub> regions modulating localization**

Proteins that localize to the nucleus and shuttle to the cytoplasm often contain nuclear localization and nuclear export signals, respectively [44-48]. TRIM5α<sub>hu</sub> and TRIM5α<sub>rh</sub> lack an obvious nuclear localization signal [54,55], nor do they contain sequences motifs predicted to function as nuclear export signals [56]. To gain some insight into the TRIM5α<sub>rh</sub> sequences that modulate nuclear localization and export, a series of TRIM5α<sub>rh</sub> mutants with deletions in N-terminal components were studied. The TRIM5α<sub>rh</sub> Δ12 and TRIM5α Δ60 proteins behaved like wild-type TRIM5α<sub>rh</sub> with respect to localization in untreated cells (Figure 5A and Table 1). However, in the LMB-treated cells, TRIM5α<sub>rh</sub> Δ12 and TRIM5α Δ60 exhibited a bright, more diffuse pattern with fewer nuclear bodies when compared with wild-type TRIM5α<sub>rh</sub> (Figure 5A and Table 1). These results indicate that neither the immediate TRIM5α<sub>rh</sub> N-terminus nor the RING domain significantly influence nuclear localization and export. By contrast, the TRIM5α<sub>rh</sub> Δ93 mutant localized to nuclear bodies and to the cytosol, even in the absence of LMB treatment (Figure 5B and Table 1). This localization pattern did not change significantly upon LMB treatment. Thus, deletion of TRIM5α<sub>rh</sub> sequences between residues 60 and 93, in the Linker 1 (L1) region of the protein, appears to decrease the efficiency of nuclear export of TRIM5α<sub>rh</sub>.

**Contribution of nuclear export of TRIM5α<sub>hu</sub> and TRIM5α<sub>rh</sub> to retroviral restriction**

To study the contribution of TRIM5α nuclear export to retroviral restriction, we treated cells stably expressing TRIM5α<sub>rh</sub> and TRIM5α<sub>hu</sub> with LMB for two hours. Then the cells were challenged with recombinant HIV-1 and N-MLV expressing GFP. Treatment with LMB continued during the incubation of the cells with virus and
overnight thereafter. LMB treatment exerted only minimal effects on the ability of TRIM5α <i>rh</i> to restrict HIV-1 infection and on the ability of TRIM5α <i>hu</i> to inhibit N-MLV infection (Figure 6).

**Discussion**

All characterized TRIM5α proteins are located in the cytoplasm of expressing cells [15,28,57-59]. Here we report the surprising observation that some TRIM5α proteins are imported into the nucleus and then exported back into the cytoplasm by a CRM1-dependent mechanism. Of interest, this transient routing through the nucleus was observed for the TRIM5α proteins of two Old World primates, and not for the TRIM5α proteins of a cow or several New World monkeys, or for the TRIMCyp protein of another New World monkey (the owl monkey). This raises the possibility that nuclear shuttling represents a property that was gained by Old World primate TRIM5α proteins after the divergence from the New World monkeys.

Our results with the GFP-PK-TRIM5α <i>rh</i> fusion protein suggest that TRIM5α <i>rh</i> is actively transported into the nucleus, as the fusion protein is well above the size limit for passive diffusion of proteins through the nuclear pore [50-52]. Nonetheless, no typical nuclear localization motif is evident on TRIM5α [54,55]. The accumulation of TRIM5α <i>hu</i> and TRIM5α <i>rh</i> in the nucleus after LMB treatment implicates a CRM1-dependent process in the export of these TRIM5α proteins from the nucleus [44-49]. However, there are no classical nuclear export motifs in TRIM5α proteins [56]. It is possible that TRIM5α utilizes unusual motifs for interacting with nuclear pore proteins. Analysis of the localization of N-terminally truncated TRIM5α <i>rh</i> mutants suggests that deletion of residues 60-93, in the linker 1 (L1) region, disrupts the nuclear export of the protein. Whether this is a result of deletion of a non-canonical nuclear export signal or an indirect effect requires further investigation. As an example of the latter effect, the linker 1 (L1) regions could mediate the association
of TRIM5αrh and TRIM5αhu with another factor that shuttles between the nuclear and cytoplasm.

Despite the accumulation of TRIM5αhu and TRIM5αrh proteins in the nucleus after LMB treatment, restriction of N-MLV and HIV-1, respectively, remained potent. Although it is possible that nuclear TRIM5αhu and TRIM5αrh can inhibit retrovirus infection, the specific recognition of the retroviral capsid, which does not enter the intact nucleus, is thought to be important for potent restriction [22,23]. A more likely explanation is
Figure 4 Localization of a GFP-PK-TRIM5α protein in leptomycin B-treated cells. HeLa cells transiently expressing the fusion constructs GFP-PK or GFP-PK-TRIM5αrh were treated with 5 ng/ml of LMB or with the equivalent concentration of DMSO for 2 hours (A). Protein expression levels of the different fusion constructs were measured by Western blot using anti-GFP antibodies (B).
Figure 5 Localization of TRIM5αrh N-terminal deletion mutants in leptomycin B-treated cells. Cf2Th cells stably expressing wild-type TRIM5αrh or the indicated deletion mutant were treated with 5 ng/ml of LMB or DMSO for two hours. Treated cells were stained using anti-HA antibodies conjugated to FITC. TRIM5αrh domains are depicted for each variant, and the numbers of the amino acid residues at the boundaries of the different domains are shown (A). L1 represents the Linker 1 region. The TRIM5αrhΔ93 protein bodies are located in the cellular nucleus (B). Cf2Th cells expressing TRIM5αrhΔ93 were stained using anti-HA antibodies conjugated to FITC (green) and propidium iodide for nuclear staining (red). A representative image is shown.
that the residual TRIM5α protein in the cytoplasm of these overexpressing cells is sufficient to inhibit virus infection. Any newly synthesized TRIM5α in these cells that has not yet entered the nucleus is potentially available for capsid interaction.

One caveat of these studies is the use of exogenously expressed TRIM5α proteins to study nuclear shuttling. When better antibodies against endogenous TRIM5α become available, the shuttling behavior of the endogenously expressed TRIM5α protein can be examined.

What might be the possible advantage of having the Old World primate TRIM5α proteins shuttle into and out of the nucleus? If these TRIM5α proteins acquire post-translational modifications or binding partners in the process, our results suggest that such acquisition is apparently not necessary for HIV-1 or N-MLV restriction. The presence of TRIM5α in the nucleus could be important for other TRIM5α functions besides retroviral restriction. For example, Old World monkey TRIM5α proteins have recently been shown to inhibit the infection of herpes simplex viruses 1 and 2 [41]. The colocalization of nuclear TRIM5α in ND10 bodies with TRIM19, which also has anti-herpes virus activity [34,39,40], might have functional importance in this respect. Future studies should shed light on these interesting possibilities.

Conclusions
Here we discovered the ability of human and rhesus TRIM5α to shuttle into and out of the nucleus. Although not essential for retroviral restriction, this novel ability of TRIM5α might be involved in other functions such as the ability of TRIM5 to trigger NF-kB [38].

Methods
Plasmid construction
The plasmids used to establish cell lines stably expressing TRIM5α variants or TRIMCyp have been previously described [8,58]. The plasmids expressing mutant TRIM5αrn proteins with N-terminal deletions were constructed by polymerase chain reaction (PCR) amplification of TRIM5 cDNA, as previously described [3]. The amplified fragments were cloned into the EcoRI and Cla I sites of the pLPCX plasmid (Stratagene). All of the TRIM5α proteins have an epitope tag from influenza hemagglutinin (HA). Human TRIM5α has the HA tag at the carboxyl terminus, and all the other TRIM5α proteins have the HA tag at the amino terminus.

Creation of cells stably expressing TRIM5α and TRIMCyp variants
Retroviral vectors encoding TRIM5α or TRIMCyp proteins were created using the pLPCX vector plasmid [3]. Recombinant viruses were produced in 293T cells by cotransfecting the pLPCX plasmids with the pVPack-GP and pVPack-VSV-G packaging plasmids (Stratagene). The pVPack-VSV-G plasmid encodes the vesicular stomatitis virus (VSV) G envelope glycoprotein, which allows efficient entry into a wide range of vertebrate cells.

Protein analysis
Cellular proteins were extracted with radioimmunoprecipitation assay (RIPA) buffer (10 mM Tris, pH 7.4; 100 mM NaCl; 1% sodium deoxycholate; 0.1% sodium dodecyl sulfate [SDS]; 1% NP-40; 2 mg of aprotinin/ml; 2 mg of leupeptin/ml; 1 mg of pepstatin A/ml; 100 mg of phenylmethylsulfonyl fluoride/ml). The cell lysates were analyzed by SDS-PAGE (10% acrylamide), followed by blotting onto nitrocellulose membranes (Amersham Pharmacia Biotech). Detection of protein by Western blotting utilized monoclonal antibodies that are specifically reactive with the HA epitope tag (Roche). Detection of proteins was performed by enhanced chemiluminescence (NEN Life Sciences Products).

Infection with recombinant viruses expressing green fluorescent protein (GFP)
Recombinant HIV-1 or N-MLV expressing GFP were prepared as described [3]. HIV-1 viral stocks were quantified by measuring reverse transcriptase (RT) activity. For infections, 3 × 10⁶ HeLa human epithelial cells or Cf2Th canine cells seeded in 24-well plates were incubated in the presence of virus for 24 hours. Cells were washed and returned to culture for 48 hours, and then

Table 1 Number of TRIM5α cytoplasmic and nuclear bodies in LMB-treated cells

| DMSO | LMB |
|------|-----|
|      | Cytoplasmic | Nuclear | Total | Cytoplasmic | Nuclear | Total |
| TRIM5αrn | 448 | 2 | 450 | 7 | 543 | 550 |
| TRIM5αrnΔ12 | 127 | 3 | 130* | 5 | 128 | 133* |
| TRIM5αrnΔ60 | 202 | 8 | 210* | 2 | 78 | 80* |
| TRIM5αrnΔ93 | 4 | 151 | 155 | 12 | 158 | 170 |

*Cytoplasmic and nuclear bodies of TRIM5αrnΔ12 and Δ60 were on average larger than bodies observed for wt TRIM5αrn and TRIM5αrnΔ93 proteins.
Figure 6 Effect of leptomycin B treatment of TRIM5α-expressing cells on retrovirus restriction. Cf2Th cells stably expressing TRIM5α_{rh} or transduced with the empty vector LPCX were challenged with increasing amounts of HIV-1-GFP in the presence of 5 ng/ml of LMB or DMSO (A). Similarly, Cf2Th cells stably expressing TRIM5α_{hu} were challenged with increasing amounts of N-MLV-GFP in the presence of 5 ng/ml of LMB or DMSO (B). TE671 cells, which naturally express TRIM5α_{hu}, were challenged with B-MLV-GFP and N-MLV-GFP in the presence of the indicated concentration of LMB or the DMSO control (C). The x-axis indicates the volume of a stock of recombinant GFP-expressing virus added to the target cells. Forty-eight hours after infection, the percentage of infected cells was measured by counting the GFP-positive cells using a flow cytometer. Similar results were obtained in three independent experiments.
subjected to FACS analysis with a FACScan (Becton Dickinson).

**Intracellular location of TRIM5 variants**

Localization of TRIM5 variants was studied as previously described [60]. Briefly, cells were grown overnight on 12-mm-diameter coverslips and fixed in 3.9% paraformaldehyde (Sigma) in phosphate-buffered saline (PBS; Cellgro) for 30 minutes. In some experiments, cells were incubated with 5 ng/ml leptotycin B (LMB) in medium for 2-10 hours prior to fixation. Cells were washed in PBS, incubated in 0.1 M glycine (Sigma) for 10 minutes, washed in PBS, and permeabilized with 0.05% saponin (Sigma) for 30 minutes. Samples were blocked with 10% donkey serum (Dako, Carpinteria, CA) for 30 minutes, and incubated for 1 hour with antibodies. HA-tagged proteins were stained using an anti-HA FITC-conjugated antibody, clone 3F10 (Roche). The TRIM19 (PML) protein was stained with an antibody against PML, sc-9863 (Santa Cruz Biotechnology, CA) and anti-goat Cy3-conjugated antibodies(Jackson Immunoresearch, PA). Subsequently, samples were mounted for fluorescence microscopy by using the ProLong Antifade Kit (Molecular Probes, Eugene, OR). Images were obtained with a BioRad Radiance 2000 laser scanning confocal microscope with Nikon 60X N.A.1.4 optics.

**Detection of TRIM5α by electron microscopy**

HeLa cells stably expressing HA-tagged TRIM5α, treated with 5 ng/ml LMB for 2 h were removed from the tissue culture dish with 5 mM EDTA in PBS, pelleted, and resuspended in a small volume of 4% paraformaldehyde in 0.2 M sodium phosphate buffer, pH 7.4. Ultrathin sections were cut at -120°C with a cryo-diamond knife. Sections were picked up from the knife with a loop dipped in a 1:1 mixture of 2.3 M sucrose and 2% methylcellulose and transferred to a carbon-coated copper grid. Grids were left floating on PBS with the section facing down. Grids were washed in PBS and blocked in 1% bovine serum albumin (BSA) in PBS for 15 min. Grids were then incubated with the anti-HA 3F10 antibody (Roche) in 1% BSA in PBS for 30 min and washed four times for 15 min in PBS. Then, the grids were incubated with Protein A-gold 10-nm particles (Jackson Immunoresearch) in 1% BSA in PBS for 20 min and washed four times for 15 min in PBS. Images were acquired using a transmission electron microscope JEOL 1200EX-80kV.

**Acknowledgements**

We thank Ms. Yvette McLaughlin and Ms. Elizabeth Carpelan for manuscript preparation and the National Institutes of Health (A0639877/JS, AI076094/JS, AI047770/17H and a Center for AIDS Research Award AI60354), the International AIDS Vaccine Initiative, the Bristol-Myers Squibb Foundation, and the late William F. McCarty-Cooper for research funding. F.D.-G. is a recipient of a K99/R00 Pathway to Independence Award from the National Institutes of Health (1K99WH081622-01), an American Foundation for AIDS Research Mathilde Kemp fellowship in basic biomedical research (1069874-3-RF#), and a Claudia Adams Barr award from the Dana-Farber Cancer Institute. We would also like to thank the James B. Pendleton Charitable Trust. T.J.H. is funded by a P50 GM082545 from the NIH.

**Author details**

1Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10461, USA. 2Department of Cancer Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA 02115, USA. 3Department of Cell and Molecular Biology, Northwestern University, Chicago, IL 60611, USA.

**Authors’ contributions**

FDG designed and performed experiments, wrote the manuscript. DEG designed and performed experiments. TJH designed and performed experiments. JS designed experiments and wrote the manuscript. All authors read and approved the final manuscript.

**Competing interests**

The authors declare that they have no competing interests.

**Received:** 1 February 2011 **Accepted:** 15 May 2011 **Published:** 15 May 2011

**References**

1. Reymond A, Meroni G, Fantozzi A, Merla G, Cairo S, Lusi L, Riganelli D, Zanara E, Messali S, Caiarusa S, et al. The tripartite motif family identifies cell compartments. *Embo J* 2001, 20:2140-2151.
2. Nicole S, Stoye JP, Saib A. TRIM family proteins: retroviral restriction and antiviral defence. *Nat Rev Microbiol* 2005, 3:799-808.
3. Stremlau M, Owens CM, Perron MJ, Kessling M, Autissier P, Sodroski J. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature* 2004, 427:848-853.
4. Hatzioannou T, Perez-Caballero D, Yang A, Cowan S, Bieniasz PD. Retrovirus resistance factors Ref1 and Lvi1 are species-specific variants of TRIM5alpha. *Proc Natl Acad Sci USA* 2004, 101:10774-10779.
5. Keckesova Z, Ylinen LM, Towers GJ. The human and African green monkey TRIM5alpha genes encode Ref1 and Lvi1 retroviral restriction factor activities. *Proc Natl Acad Sci USA* 2004, 101:10780-10785.
6. Perron MJ, Stremlau M, Song B, Ulm W, Mulligan RC, Sodroski J. TRIM5alpha mediates the postentry block to N-tropic murine leukemia viruses in human cells. *Proc Natl Acad Sci USA* 2004, 101:11827-11832.
7. Yap MW, Nicole S, Lynch C, Stoye JP. Trim5alpha protein restricts both HIV-1 and murine leukemia virus. *Proc Natl Acad Sci USA* 2004, 101:10796-10791.
8. Song B, Javanbakht H, Perron M, Park DH, Stremlau M, Sodroski J. Retrovirus restriction by TRIM5alpha variants from Old World and New World primates. *J Virol* 2005, 79:3930-3937.
9. Nicole 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 USA* 2004, 101:13324-13328.
10. 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.
11. Song B, Gold B, O’Huigin C, Javanbakht H, Li X, Stremlau M, Winkleiter C, Dean M, Sodroski J. The B30.2(SPRY) domain of the retroviral restriction factor TRIM5alpha exhibits lineage-specific length and sequence variation in primates. *J Virol* 2005, 79:6111-6121.
12. Kono K, Bozek K, Domingues FS, Shoda T, Nakayama EE. Impact of a single amino acid in the variable region 2 of the Old World monkey TRIM5alpha SPRY (B30.2) domain on anti-human immunodeficiency virus type 2 activity. *Virology* 2009, 388:160-168.
13. Sebastian S, Grutter C, de Castilla CS, Pertel T, Okvani S, Guttert MG, Luban J. An invariant surface patch on the TRIM5alpha PRYSPRY domain is required for retroviral restriction but dispensable for capsid binding. *J Virol* 2009, 83:3365-3373.
The TRIM5alpha B-box 2 domain promotes cooperative DNA viruses and viral proteins that interact with PML nuclear bodies. J Virol 2005, 79:1573-78.

Nakayama EE, Shioda T: Anti-retroviral activity of TRIM5 alpha. Rev Med Virol 2010, 20:77-92.

Xu D, Holko M, Sadler AJ, Scott B, Higashiyama S, Berkofsky-Fessler W, McConnell MJ, Pandolfi PP, Licht JD, Williams BR: Promyelocytic leukemia zinc finger protein regulates interferon-mediated innate immunity. Immunity 2009, 30:802-816.

Carragena L, Parise MC, Ringead M, Chebli-Alix MK, Hazan U, Nosile S: Implication of TRIM alpha and TRIMCyp in interferon-induced anti-retroviral restriction activities. Retrovirology 2008, 5:59.

Nishikawa N, Yoshida M, Fujisawa D, Nishikawa M, Horiouchi S, Beppu T: Leptomycin B targets a regulatory cascade of cIAP1, a fission yeast nuclear protein, involved in control of higher order chromosome structure and gene expression. J Biol Chem 1994, 269:6320-6324.

Watanabe M, Fukuda M, Yoshida M, Yanagida M, Nishida E: Involvement of CRM1, a nuclear export receptor, in mRNA export in mammalian cells and fission yeast. Genes Cells 1999, 4:299-307.

Wolf B, Sangler J, Wang Y: Leptomycin B is an inhibitor of nuclear export: inhibition of nuclear-cytoplasmic translocation of the human immunodeficiency virus type 1 (HIV-1) Rev protein and Rev-dependent mRNA. Chem Biol 1997, 4:139-147.

Fornedel M, Ohno M, Yoshida M, Matrajt IW: CRM1 is an export receptor for leucine-rich nuclear export signals. Cell 1997, 90:1051-1060.

Kuerten S, Ohno M, Matrajt IW: Nucleocytoplasmic transport: Ran, beta and beyond. Trends Cell Biol 2001, 11:497-503.

Kudo N, Wolf B, Sekimoto T, Schreiner EP, Yonedo Y, Yanagida M, Horinouchi S, Yoshida M: Leptomycin B inhibition of signal-mediated nuclear export by direct binding to CRM1. Exp Cell Res 1998, 242:540-547.

Mohr D, Frey S, Fischer T, Gutter J, Gerlich D: Characterisation of the passive permeability barrier of nuclear pore complexes. EMBO J 2000, 20:2541-2553.

Stewart M, Baker RP, Bayliss R, Clayton L, Grant RP, Littlewood T, Matsuura Y: Molecular mechanism of translocation through nuclear pore complexes during nuclear protein import. FEBS Lett 2001, 498:145-149.

Daboussi MC, Frankie WW: Determination of the intracellular state of soluble macromolecules by gel filtration in vivo in the cytoplasm of amphibian oocytes. J Cell Biol 1986, 102:2006-2014.

Heibert SW, Lamb RA: Cell surface expression of glycosylated, nonglycosylated, and truncated forms of a cytoplasmic protein pyruvate kinase. J Cell Biol 1988, 107:865-876.

Brameier M, Kring M, MacCallum RM: NuclPred—predicting nuclear localization of proteins. Bioinformatics 2003, 21:1559-1563.

Nar R, Rost B: LOC3D: annotate sub-cellular localization for protein structures. Nucleic Acids Res 2003, 31:3337-3340.

La Cour T, Kiemer L, Molgaard A, Gupta R, Skriver K, Brunak S: Analysis and prediction of leucine-rich nuclear export signals. Protein Eng Des Sel 2004, 17:527-536.

Campbell EM, Perez O, Anderson JL, Hope TJ: Visualization of a proteasome-independent intermediate during restriction of HIV-1 by rhesus TRIM5alpha. J Cell Biol 2008, 180:549-561.

Diaz-Griffero F, Kar A, Lee M, Mestlau M, Poeschla E, Sodroski J: Comparative requirements for the restriction of retrovirus infection by TRIMalpha and TRIMCyp. Virology 2007.

Campbell EM, Perez O, Anderson JL, Hope TJ: Visualization of a proteasome-independent intermediate during restriction of HIV-1 by rhesus TRIM5alpha. J Cell Biol 2008, 180:549-561.

Modulation of Retroviral Restriction and Proteasome Inhibitor-resistant Turnover by Changes in the TRIM5alpha B-box 2 Domain. J Virol 2007.

Diaz-Griffero F, Perron M, Pierson M, Geese-Estrada K, Hanna R, Mailard PV,充足 Lui, Sodroski J: A human TRIM5alpha B30.2/SPRY domain mutant gains the ability to restrict and prematurely uncoat B-tropic murine leukemia virus. Virology 2008.

Perron MJ, Mestlau M, Lee M, Javanbakht H, Song B, Sodroski J: The human TRIM5alpha restriction factor mediates accelerated uncoating of the N-tropic murine leukemia virus capsid. J Virol 2007, 81:2136-2148.

Everett RD: DNA viruses and viral proteins that interact with PML nuclear bodies. Oncogene 2001, 20:7266-7273.

Negorev D, Mau G: Cellular proteins localized at and interacting within ND10/PML nuclear bodies/PDs suggest functions of a nuclear depot. Oncogene 2001, 20:7234-7242.

Mau G: Nuclear domain 10, the site of DNA virus transcription and replication. Bioessays 1998, 20:660-667.

Everett RD, Mau G: HSV-1 IE protein Vmw110 causes redistribution of PML. Exp Cell Res 1994, 133:5062-5069.

Bjorndal AS, Szekely L, Elgh F: Ebola virus infection inversely correlates with the overall expression levels of promyelocytic leukemia (PML) protein in cultured cells. BMC Microbiol 2003, 3:6.

Bonilla WV, Pinscherwe DD, Klenemann P, Rousson V, Gaboli M, Pandolfi PP, Zinkenberg RM, Salvalato MS, Hengartner H: Effects of promyelocytic leukemia protein on virus-host balance. J Virol 2002, 76:3810-3818.

Djavan M, Rodas J, Lukashechik IS, Horejsh D, Pandolfi PP, Borden KL, Salvalato MS: Role of the promyelocytic leukemia protein PML in the interferon sensitivity of lymphocytic choriomeningitis virus. J Virol 2001, 75:6204-6208.

Taren SJ, Emerman M: Human TRIM5alpha has additional activities that are uncoupled from retroviral capsid recognition. Virology 2011, 409:113-120.

Kurahara A, Boaz K, Shioda T, Nakayama EE: A single amino acid substitution of the human immunodeficiency virus type 1 capsid protein affects viral sensitivity to TRIM5alpha. Retrovirology 2010, 7:58.

Reszka N, Zhou C, Song B, Sodroski JG, Knipe DM: TRIM5alpha protein in cultured cells. J Virol 2002, 76:12866-12876.

Diaz-Griffero F, Horschander SA, Brouatch J: Endocytosis is a critical step in entry of subgroup B avian leuks virus. J Virol 2002, 76:9664-9668.