The Contribution of RING and B-box 2 Domains to Retroviral Restriction Mediated by Monkey TRIM5α*

Hassan Javanbakht†, Felipe Diaz-Griffero‡, Matthew Stremlau§, Zhihai Si∥, and Joseph Sodroski†‡¶

From the †Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Department of Pathology, Division of AIDS, Harvard Medical School and the ‡Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts 02115

TRIM5α is a cytoplasmic protein that mediates a post-entry block to infection by some retroviruses. TRIM5α contains a tripartite motif (TRIM), which includes RING, B-box 2, and coiled-coil domains, and a C-terminal B30.2 (SPRY) domain. We investigated the contribution of the RING and B-box 2 domains to the antiretroviral activity of rhesus monkey TRIM5α (TRIM5αrh), which potently restricts infection by human immunodeficiency virus, type 1 (HIV-1) and simian immunodeficiency virus of African green monkeys (SIVagm). Disruption of the RING domain caused mislocalization of TRIM5αrh so that the cytoplasmic level of the protein was decreased compared with that of the wild-type protein. Nonetheless, partial ability to restrict HIV-1 and SIVagm was retained by the RING domain mutants. By contrast, although TRIM5αrh mutants with disrupted B-box 2 domains were efficiently expressed and correctly localized to the cytoplasm, antiretroviral activity was absent. The B-box 2 mutants colocalized and associated with wild-type TRIM5αrh and exerted dominant-negative effects on the antiretroviral activity of the wild-type protein. Taken together with other data, these results indicate that functionally defective TRIM5αrh molecules that retain a coiled coil can act as dominant-negative inhibitors of wild-type TRIM5αrh function. The RING domain of TRIM5α is not absolutely required for retrovirus restriction but can influence cytoplasmic levels of the protein and thus indirectly alter function. The B-box 2 domain, by contrast, appears to be essential for efficient retrovirus restriction.

Host cell factors influence the infectivity of retroviruses. Following entry into the host cell, uncoating of the viral core, reverse transcription, nuclear access, and integration of the viral DNA into the host genome must occur to establish a permanent infection (1, 2). Early, post-entry restrictions to retrovirus infection can determine tropism at the species level. HIV-1 and SIVmac encounter a post-entry block in Old World monkeys, whereas SIVmac is blocked in most New World monkey cells (3–6). These species-specific restrictions occur prior to or concurrent with reverse transcription; at most, low levels of early reverse transcripts are made in restricted cells (3, 5, 7, 8). The viral determinant of susceptibility to these blocks is the capsid protein (7, 9–13). The early restriction to HIV-1 and SIV is mediated by dominant host factors, the activity of which can be titrated by the introduction of virus-like particles containing proteolytically processed capsid proteins of the restricted viruses (7, 12, 14–18). Thus, in the cells of specific monkey species, host restriction factors apparently interact, directly or indirectly, with the HIV-1 or SIV capsid and prevent its progression along the infectious pathway.

A genetic screen identified TRIM5α as a major restriction factor in monkey cells that acts on HIV-1 and, to a lesser extent, on SIVmac (19). The expression of rhesus monkey TRIM5α was shown to be sufficient to confer potent resistance to HIV-1 infection in otherwise susceptible cells. Moreover, TRIM5α was necessary for the maintenance of the block to the early phase of HIV-1 infection in Old World monkey cells, as demonstrated by interference with TRIM5α expression in these cells. Differences among the sequences of TRIM5α proteins of primate species account for the different abilities to restrict infection by particular retroviruses (6, 20–23).

TRIM5 is a member of a family of proteins that contain a tripartite motif, hence the designation TRIM (24). The tripartite motif includes a RING domain, B-box 2 domain and coiled-coil (cc) domain; TRIM proteins have also been called RBCC proteins. TRIM proteins exhibit the propensity to assemble into cytoplasmic or nuclear bodies (24). Many cytoplasmic TRIM proteins contain a C-terminal B30.2 or SPRY domain. Differential splicing of the TRIM5 primary transcript gives rise to the expression of several isoforms of the protein product. The TRIM5α isoform is the largest product (∼493 amino acid residues) and contains the B30.2(SPRY) domain. The B30.2(SPRY) domain of rhesus monkey TRIM5α is essential for anti-HIV-1 activity (19). Moreover, the difference in the anti-HIV-1 potency of rhesus and human TRIM5α proteins is determined by B30.2(SPRY) sequences (25–27). An intact RING domain also contributes, either directly or indirectly, to the anti-HIV-1 activity of rhesus monkey TRIM5α (19).

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† Both authors contributed equally to this work.
‡ Supported by a fellowship from the Canadian Institutes of Health Research.
§ To whom correspondence should be addressed: Dana-Farber Cancer Institute, 44 Binney St., JFB 824, Boston, MA 02115. Tel.: 617-632-3371; Fax: 617-632-4338; E-mail: joseph_sodroski@dfci.harvard.edu.

1 The abbreviations used are: HIV-1, human immunodeficiency virus, type 1; SIV, simian immunodeficiency virus; SIVmac, SIV of African green monkeys; SIVagm, SIV of rhesus macaques; HA, hemagglutinin; VSV, vesicular stomatitis virus; GFP, green fluorescence protein; FACS, fluorescence activated cell sorting; RIPA, radioimmunoprecipitation assay buffer; PBS, phosphate-buffered saline; PRL, primary rhesus lung.

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contribution of the RING, B-box 2, and coiled-coil domains to the antiviral activity of rhesus monkey TRIM5α.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—The plasmids expressing the wild-type and mutant TRIM5α proteins were constructed using PCR-directed mutagenesis. The TRIM5α cDNA was PCR-amplified (19) and digested with EcoRI and ClaI, whose sites were introduced in each of the PCR primers. These fragments were cloned into the EcoRI and ClaI site of pcDNA3.1 (Invitrogen). The plasmid expressing TRIM5α-V5 was constructed by inserting a PCR-amplified TRIM5α cDNA into the Viral Power plasmid using directional TOPO® cloning (Invitrogen).

**Creation of Cells Stably Expressing TRIM5α Variants**—Retroviral vectors encoding wild-type or mutant TRIM5α proteins were created using the pLPCX vector (19). The pLPCX vectors contain only the amino acid-coding sequence of the TRIM5α cDNA. The TRIM5α proteins encoded by the pLPCX vectors possess C-terminal epitope tags derived from influenza hemagglutinin (HA). 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.

TRIM5α-V5 was made using the Viral Power system (Invitrogen). The TRIM5α-V5 protein possesses a V5 C-terminal epitope tag. Recombinant lentiviruses were produced according to the manufacturer’s protocol (Invitrogen). The resulting virus particles were used to transduce ~1 × 10⁶ HeLa cells in the presence of 5 μg/ml Polybrene. Cells were selected in either 1 μg/ml puromycin (Sigma) or 1 μg/ml puromycin and 2 μg/ml blasticidin (Invitrogen).

**Infection with Viruses Expressing GFP**—Recombinant HIV-1, SIVmac and SIVagm expressing GFP were prepared as described previously (6, 19). HIV-1 viral stocks were quantified by measuring reverse transcriptase activity. For infections, 3 × 10⁴ HeLa cells seeded in 24-well plates were incubated in the presence of virus for 24 h. Cells were washed and returned to culture for 48 h and then subjected to FACS analysis with a FACScan (BD Biosciences).

**Protein Analysis**—Cellular proteins were extracted with radioimmune precipitation assay (RIPA) buffer (10 mM Tris, pH 7.4, 100 mM NaCl, 1% sodium deoxycholate, 0.1% SDS, 1% Nonidet P-40, 2 mg/ml aprotinin, 2 mg/ml leupeptin, 1 mg/ml pepstatin A, 100 μg/ml phenylmethylsulfonyl fluoride). The cell lysates were analyzed by SDS-PAGE (10% acrylamide), followed by blotting onto nitrocellulose membranes (Amersham Biosciences). Detection of protein by Western blotting utilized monoclonal antibodies that are specifically reactive with the HA (Roche Applied Science) or V5 (Invitrogen) epitope tags, and monoclonal antibodies to β-actin (Sigma). Detection of proteins was performed by enhanced chemiluminescence (PerkinElmer Life Sciences), using the following secondary antibodies obtained from Amersham Biosciences: anti-mouse (for V5 and β-actin) and anti-rat (for HA).

**Colocalization Experiments**—Colocalization was studied as previously described (28). 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 min. Cells were washed in PBS, incubated in 0.1% glycine (Sigma) for 10 min, washed in PBS, and permeabilized with 0.05% saponin (Sigma) for 30 min. Samples were blocked with 10% donkey serum (Dako, Carpinteria, CA) for 30 min, and incubated for 1 h with antibodies. The anti-HA fluorescein isothiocyanate-conjugated 3F10 antibody (Roche Applied Science) and anti-V5 Cy3-conjugated antibody (Sigma) were used to stain HA- or V5-tagged TRIM5α proteins, respectively. Subsequently, samples were mounted for fluorescence microscopy by using the ProLong Antifade Kit for fluorescence microscopy.
Immunoprecipitations—At 48 h after transfection, 293T cells were removed from the plate and washed with PBS. 293T cells from nearly confluent 100-mm plates were lysed in 500 μl of RIPA buffer. Insoluble material was pelleted at 22,000 × g for 2 h, and the supernatant was used for immunoprecipitation. Equal amounts of protein (200–500 μg, as determined by the Bio-Rad assay) were incubated with 30 μl of protein A-Sepharose (50%) and 5 μl of anti-HA antibody (Roche Applied Science) for 2 h at 4 °C. The immunoprecipitates were then washed three times with RIPA buffer and twice with PBS. After the final supernatant was removed, 30 μl of 2× sample buffer (120 mM Tris-HCl, pH 6.8, 20% glycerol, 4% SDS, and 0.02% bromphenol blue) was added, and the precipitate was boiled for 5 min to release the precipitated proteins. After centrifugation, the resulting supernatant was analyzed by SDS-PAGE and Western blotting.

RESULTS

Role of the RING and B-box 2 Domains in Retrovirus Restriction—TRIM5αrh contains a tripartite motif that includes a RING (residues 15–59) and B-box 2 (residues 97–129) domains (19, 24, 29–31). To investigate the role of these domains in retrovirus restriction, we generated rhesus monkey TRIM5α mutants with alterations affecting either one or both of these domains (Fig. 1A). The TRIM5αrh-HA Δ93 protein lacks the RING domain, and the TRIM5αrh-HA Δ132 protein lacks the RING and B-box 2 domains. We also altered specific cysteines and histidines within these domains; these residues are critical for zinc binding and are thought to contribute to the proper folding of these domains (29). In the C15A/C18A mutant, two key cysteines within the RING domain of TRIM5αrh-HA were altered to alanines. In the C97A/H100A mutant, cysteine and histidine residues conserved among B-box 2 domains were changed to alanines. The wild-type and mutant TRIM5αrh-HA proteins were expressed stably in HeLa cells (Fig. 1B). The TRIM5αrh-HA Δ93, C15A/C18A, and C97A/H100A mutants were expressed at levels comparable to that of the wild-type TRIM5αrh-HA protein. The TRIM5αrh-HA Δ132 protein was expressed at higher levels than that of the wild-type protein.

The HeLa cells expressing the wild-type and mutant TRIM5αrh-HA proteins were incubated with recombinant retroviruses (HIV-1-GFP, SIVmac-GFP, and SIVagm-GFP) pseudotyped with the vesicular stomatitis virus G glycoprotein.
and expressing GFP. The wild-type TRIM5αrH-HA protein potently restricted HIV-1-GFP infection (Fig. 2A). Cells expressing the C15A/C18A or Δ93 mutants were less susceptible to HIV-1-GFP infection than the control cells transduced the empty pLPCX vector, although the level of restriction observed was significantly less potent than that observed in the cells expressing wild-type TRIM5αrH-HA. HIV-1-GFP infection of cells expressing the TRIM5αrH-HA Δ132 and C97A/H100A proteins was slightly more efficient than that of cells transduced with the empty pLPCX vector. We conclude that disruption of the RING domain partially attenuates the HIV-1-restricting activity of TRIM5αrH, whereas disruption of the B-box 2 domain completely eliminates HIV-1 restriction.

One explanation for the partial anti-HIV-1 activity of the TRIM5αrH-HA RING domain mutants is functional complementation by endogenous TRIM5α. It is possible, however, that another TRIM protein in canine cells complemented the activity of the RING domain-deleted TRIM5αrH mutant.

The rhesus monkey TRIM5α protein potently blocks infection by SIVagm (13, 15). The activities of the mutant TRIM5αrH-HA proteins in restricting SIVagm-GFP infection were similar to those observed for HIV-1-GFP infection (Fig. 2B). The TRIM5αrH-HA Δ93 and C15A/C18A mutants slightly inhibited SIVagm-GFP infection, compared with the level of infection observed in control cells transduced with the pLPCX vector. By contrast, cells expressing the Δ132 and C97A/H100A mutants were infected more efficiently than the control cells transduced with the empty vector. As was observed for restriction of HIV-1, restriction of SIVagm by rhesus monkey TRIM5α apparently requires the B-box 2 domain and is potentiated by the RING domain. The moderate inhibition of SIVagm infection previously observed for the rhesus monkey TRIM5α protein.

FIG. 3. Anti-HIV-1 activity of wild-type and mutant TRIM5αrH-HA proteins in canine cells. Cf2Th canine thymocytes expressing the wild-type or C15A/C18A TRIM5αrH-HA protein, or control Cf2Th cells transduced with the empty pLPCX vector (vector), were incubated with various amounts of HIV-1-GFP. Infected, GFP-positive cells were counted by FACS. The results of a typical experiment are shown. Similar results were obtained in three independent experiments.

FIG. 4. Subcellular localization of wild-type and mutant TRIM5αrH. HeLa cells stably expressing the HA-tagged wild-type or mutant TRIM5αrH proteins were fixed and stained using a fluorescein isothiocyanate-conjugated anti-HA antibody, as described under “Experimental Procedures.” Representative confocal microscopic images are shown of the HeLa cells transduced with the empty pLPCX vector (vector) (A), or expressing wild-type TRIM5αrH-HA (B), TRIM5αrH-HA Δ93 (C), TRIM5αrH-HA C15A/C18A (D), TRIM5αrH-HA Δ132 (E), or TRIM5αrH-HA C97A/H100A (F).

FIG. 5. Coexpression of wild-type and mutant TRIM5αrH-HA proteins with TRIM5αrH-V5. HeLa cells stably expressing the wild-type and mutant TRIM5αrH-HA proteins, or control HeLa cells transduced with the empty pLPCX vector, were stably transduced with a vector expressing TRIM5αrH-V5. The TRIM5αrH-V5 protein is tagged with a C-terminal V5 tag. The lysates of these cells were Western blotted and probed with an antibody directed against HA (upper panel), V5 (middle panel), or β-actin (lower panel).
(19) was abrogated by disruption of either the RING or B-box 2 domain (Fig. 2C).

Subcellular Localization of TRIM5α_H9251_HA Mutants—The subcellular localization of the wild-type and mutant TRIM5α_H9251_HA proteins was examined by staining cells with an antibody directed against the HA epitope tag. As has been described for several TRIM proteins (24), TRIM5α_H9251_HA formed cytoplasmic bodies and also exhibited diffuse cytoplasmic staining (Fig. 4B). Compared with the wild-type TRIM5α_H9251_HA protein, the TRIM5α_H9251_HA Δ93 and C15A/C18A mutants exhibited less intense cytoplasmic staining. The bodies formed by TRIM5α_H9251_HA Δ93 were larger than those of the wild-type TRIM5α_H9251 protein, and were found in the nucleus as well as the cytoplasm (Fig. 4C). Cells expressing TRIM5α_H9251_HA C15A/C18A exhibited bodies that were larger and more numerous than those formed by the wild-type protein; the bodies formed by the C15A/C18A mutant were located in both the nucleus and cytoplasm (Fig. 4D). Thus, disruption of the RING domain causes significant changes in the subcellular localization of TRIM5α_H9251; notably, decreases in the levels of diffuse cytoplasmic staining were observed for these mutants compared with that of the wild-type TRIM5α_H9251 protein.

The TRIM5α_H9251_HA Δ132 mutant lacking the RING and B-box 2 domains exhibited diffuse nuclear and cytoplasmic staining; the intensity of staining was consistent with the high steady-state level of expression observed for this protein (Fig. 4E). The TRIM5α_H9251_HA C97A/H100A mutant exhibited a diffuse pattern of cytoplasmic staining with occasional small speckles evident (Fig. 4F). Thus, disruption of the B-box 2 domain, either alone or in combination with the RING domain,
alters the pattern of TRIM5αR localization in cells; nonetheless, the cytoplasmic levels of the B-box 2 mutants were equal to or greater than that of the wild-type TRIM5αR protein.

**Dominant-negative Activity of TRIM5α Mutants**—We wished to investigate whether the RING or B-box 2 mutants might exhibit a dominant-negative phenotype with respect to the antiviral function of wild-type TRIM5αR. To this end, we transduced the HeLa cell lines expressing the wild-type or mutant TRIM5αR-HA proteins with a lentivirus vector expressing a wild-type TRIM5αR protein with a V5 epitope tag at the C terminus (TRIM5αR-V5). The expression level of TRIM5αR-V5 varied in the transduced cell lines (Fig. 5), possibly reflecting the effect of the initially expressed TRIM5αR-HA proteins on transduction efficiency or on the ability of the cells to tolerate TRIM5αR-V5 expression.

To determine whether the expression of the mutant TRIM5αR-HA proteins might affect TRIM5αR-V5 function, the cell lines were incubated with different amounts of HIV-1-GFP, SIVagm-GFP, and SIVmac-GFP. For all three virus infections, the percentage of GFP-positive cells in the cell lines initially containing the pLPCX vector and transduced with the vector expressing wild-type TRIM5αR-V5 was lower than that in control cells expressing only the vector (Fig. 6, A–C). For all three viruses, the percentages of GFP-positive cells were comparable for the cells initially containing the pLPCX vector and the cells initially containing the RING domain TRIM5αR-HA mutants, both of which were subsequently transduced with the vector expressing TRIM5αR-V5. These results indicate that the RING domain mutants exert minimal dominant-negative effects on the ability of TRIM5αR-V5 to restrict HIV-1, SIVagm, or SIVmac infection. By infection, all three viruses was more efficient in the cells initially expressing the TRIM5αC-HA Δ132 and C97A/H100A mutants and subsequently transduced with the vector expressing TRIM5αR-V5, compared with the infection of cells initially containing the empty pLPCX vector and transduced with the vector expressing TRIM5αR-V5. In the case of SIVagm and SIVmac infections, the percentages of GFP-positive cells in the Δ132- and C97A/H100A-expressing cells transduced with TRIM5αR-V5 were similar to those seen in the control cells containing only the empty pLPCX vector. We conclude that the TRIM5αC-HA Δ132 and C97A/H100A mutants can inhibit the antiretroviral activity of the wild-type TRIM5αR-V5 protein in a dominant-negative manner.

We examined the subcellular localization of the wild-type and mutant TRIM5αR-HA proteins in the cells expressing the wild-type TRIM5αR-V5 protein by confocal microscopy (Fig. 7). The HA and V5 epitope-tagged proteins were detected by indirect immunofluorescence, using anti-HA-fluorescein isothiocyanate and anti-V5-Cy3, respectively. Thus, the TRIM5αR-HA proteins exhibit green fluorescence (left panels, Fig. 7) and the TRIM5αR-V5 proteins exhibit red fluorescence (middle panels, Fig. 7). The right panels represent overlays of the green and red fluorescence from the HA-tagged and V5-tagged TRIM5αR proteins, respectively. The TRIM5αR-V5 expressed in the cells with the empty pLPCX vector exhibited diffuse cytoplasmic staining as well as small, scattered cytoplasmic bodies (Fig. 7A), as seen previously for the TRIM5αR-HA protein (Fig. 4B). As expected, the wild-type TRIM5αR-HA and TRIM5αR-V5 proteins colocalized in the cytoplasm (Fig. 7B). In cells coexpressing TRIM5αR-HA Δ93 and TRIM5αC-V5, both proteins exhibited diffuse cytoplasmic staining (Fig. 7C). Both proteins also colocalized in nuclear bodies, indicating that the coexpression of the TRIM5αR-HA Δ93 mutant causes relocalization of some of the wild-type TRIM5αR-V5 protein to these nuclear structures. Minimal colocalization was observed for the TRIM5αR-HA C15A/C18A mutant and the TRIM5αR-V5 protein (Fig. 7D). In cells coexpressing TRIM5αR-HA Δ132 and TRIM5αR-V5, the former protein exhibited diffuse nuclear and cytoplasmic staining (Fig. 7E). The wild-type TRIM5αR-V5 protein in these cells did not form cytoplasmic bodies, but maintained a diffuse cytoplasmic pattern of staining that colocalized with the cytoplasmic portion of the TRIM5αR-V5 Δ132 protein. The TRIM5αR-HA C97A/H100A and wild-type TRIM5αR-V5 proteins colocalized in a diffuse cytoplasmic pattern of staining (Fig. 7F). As was seen in cells expressing the TRIM5αR-HA Δ132 protein, the wild-type TRIM5αR-V5 protein did not form cytoplasmic bodies in the cells coexpressing TRIM5αR-HA C97A/H100A.

![Image](http://www.jbc.org/Downloadedfrom)
The above studies suggest that the TRIM5α<sub>Δ93</sub>-HA mutants with disrupted B-box 2 domains exhibit dominant-negative phenotypes. To examine this dominant-negative activity in a primary monkey cell that naturally expresses TRIM5α<sub>Δ93</sub>, primary rhesus lung (PRL) fibroblasts were transduced with the empty pLPCX vector or expressing the TRIM5α<sub>Δ93</sub>-HA Δ93 or TRIM5α<sub>Δ132</sub> proteins. PRL cells have been previously shown to restrict HIV-1 infection potentially; furthermore, this restriction can be relieved by TRIM5α-directed siRNA or by the expression of the TRIM5α<sub>y</sub> isofrom (19). The expression of the TRIM5α-HA Δ132 protein in the PRL cells significantly increased the susceptibility of these cells to HIV-1 infection (Fig. 8). By contrast, expression of the TRIM5α<sub>Δ93</sub>-HA Δ93 protein resulted in only a minor increase in the level of HIV-1 infection, compared with control PRL cells transduced with the empty pLPCX vector (Fig. 8). Thus, the TRIM5α<sub>Δ93</sub>-HA Δ132 protein exhibits dominant-negative activity in interfering with the HIV-1-restricting function of the wild-type rhesus monkey TRIM5α protein.

The basis for the partial loss of antiretroviral activity of TRIM5α<sub>Δ93</sub>-HA mutants is known. Because TRIM5α<sub>y</sub> is known to act between the introdution of the viral capsid into the cytoplasm and reverse transcription (19), cytoplasmic levels of TRIM5α are likely important for antiretroviral activity of TRIM5α<sub>Δ93</sub> associated with RING domain disruption is unknown. Because TRIM5α<sub>y</sub> is known to act between the introduction of the viral capsid into the cytoplasm and reverse transcription (19), cytoplasmic levels of TRIM5α are likely important for antiretroviral function. It is probable that at least part of the decrease in HIV-1-suppressive activity of the TRIM5α<sub>Δ93</sub> RING domain mutants is due to mislocalization of the mutant proteins in the cell. Levels of diffusely staining,
The phenotypes of the TRIM5αΔB2 mutants in which the B-box 2 domain is disrupted distinctly differ from those of the RING domain mutants. Deletion of the RING and B-box 2 domains, or disruption of only the B-box 2 domain, completely eliminated the antiretroviral activity of TRIM5αΔB2. These mutants were expressed efficiently, at least as well as wild-type TRIM5αΔB2, and localized in the cytoplasm. Apparently, the B-box 2 plays an important role in retroviral restriction mediated by TRIM5α, although further studies are required to elucidate this role. In promyelocytic protein (TRIM19), disruption of either of the two B-boxes interferes with nuclear body formation without affecting homo-oligomerization. In contrast, the structural integrity of the B-box contributes to the self-association of the ret finger protein TRIM27. The B-box 2 mutants associate with the wild-type TRIM5αΔB2 protein, suggesting that these may retain the ability to oligomerize. The B-box 2 potentially contributes to the TRIM5αΔB2 interaction with the retroviral capsid or host cofactors for restriction. B-box domains have been reported to be important for the association of TRIM1, TRIM18, and TRIM20 with elements of cell signaling pathways (33, 34).

Our observation that the TRIM5αΔB2 Δ132 but not the Δ297 mutant associates with wild-type TRIM5αΔB2 implies that the coiled-coil domain makes a major contribution to TRIM5αΔB2 self-association. Coiled-coil domains have been implicated in the ability of other TRIM proteins to form homo-oligomers (24, 33–35).

Our results suggest that B-box 2 mutants can interfere with the antiretroviral activity of the wild-type TRIM5αΔB2 protein in a dominant-negative manner. The mild increase in the susceptibility of HeLa cells expressing the B-box 2 mutants to HIV-1 and SIV infection may reflect dominant-negative inhibition of the modest antiretroviral activity of the endogenous human TRIM5α in these cells (19). The TRIM5γΔB2 isoform, which lacks the B30.2/SPRY domain and exhibits no antiretroviral activity, has been shown to exhibit dominant-negative activity (19). TRIM5γB2 can associate with TRIM5αΔB2 when the two proteins are coexpressed in cells (data not shown). The ability of TRIM5 variants to associate with and to interfere with the antiretroviral activity of wild-type TRIM5α may be related. Of interest, all of the TRIM5 variants that retain these two properties contain a coiled-coil domain.

The levels of expression of TRIM5αΔB2 Δ132 and C97A/H100A mutants were greater than or equal to those of the wild-type TRIM5αΔB2 in the cells where restriction by the wild-type protein was relieved. These levels of expression likely contribute to the ability of the dominant-negative mutants to compete with the wild-type TRIM5αΔB2 protein for factors, binding to which is necessary for antiviral function. Candidates for such factors include the viral capsid, TRIM5α itself, and cellular cofactors. Future studies will be directed toward a more complete understanding of the importance of specific elements of the tripartite motif of TRIM5α in retrovirus restriction.

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Hassan Javanbakht, Felipe Diaz-Griffero, Matthew Stremlau, Zhihai Si and Joseph Sodroski

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