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Abstract

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ABSTRACT
Host restriction factor TRIM5 inhibits retroviral transduction in a species-specific manner by binding to and destabilizing the retroviral capsid lattice before reverse transcription is completed. However, the restriction mechanism may not be that simple since TRIM5 E3 ubiquitin ligase activity, the proteasome, autophagy, and TAK1-dependent AP-1 signaling have been suggested to contribute to restriction. Here, we show that, among a panel of seven primate and Carnivora TRIM5 orthologues, each of which has potential for potent retroviral restriction activity, all activated AP-1 signaling. In contrast, TRIM family paralogues most closely related to TRIM5 did not. While each primate species has a single TRIM5 gene, mice have at least seven TRIM5 homologues that cluster into two groups, Trim12a, -b, and -c and Trim30a, -b, -c, and -d. The three Trim12 proteins activated innate immune signaling, while the Trim30 proteins did not, though none of the murine Trim5 homologues restricted any of a panel of cloned retroviruses. To determine if any mouse TRIM5 homologues had potential for restriction activity, each was fused to the human immunodeficiency virus type 1 (HIV-1) CA binding protein cyclophilin A (CypA). The three Trim12-CypA fusions all activated AP-1 and restricted HIV-1 transduction, whereas the Trim30-CypA fusions did neither. AP-1 activation and HIV-1 restriction by the Trim12-CypA fusions were inhibited by disruption of TAK1. Overall then, these experiments demonstrate that there is a strong correlation between TRIM5 retroviral restriction activity and the ability to activate TAK1-dependent innate immune signaling.

IMPORTANCE
The importance of retroviruses for the evolution of susceptible host organisms cannot be overestimated. Eight percent of the human genome is retrovirus sequence, fixed in the germ line during past infection. Understanding how metazoa protect their genomes from mutagenic retrovirus infection is therefore of fundamental importance to biology. TRIM5 is a cellular protein that protects host genome integrity by disrupting the retroviral capsid as it transports viral nucleic acid to the host cell nucleus. Previous data suggest that innate immune signaling contributes to TRIM5-mediated restriction. Here, we show that activation of innate immune signaling is conserved among primate and carnivore TRIM5 orthologues and among 3 of the 7 mouse Trim5 homologues and that such activity is required for TRIM5-mediated restriction activity.

When a retrovirus fuses its membrane with a susceptible target cell, it releases the virion core into the host cell cytoplasm (1). The core is composed of a capsid (CA) protein lattice, within which is the viral RNA. The viral RNA is reverse transcribed into cDNA by the viral reverse transcriptase (RT). Via a poorly characterized mechanism, the viral cDNA is transferred to the host cell nucleus. The viral integrase protein (IN) then ligates the viral cDNA into the host chromosomal DNA to generate the provirus, thus completing the transduction process.

Host species that are susceptible to retroviral infection encode factors that block retroviral transduction, perhaps because these early events in the retroviral life cycle result in mutation of the host genome that is potentially catastrophic. The genes encoding these antiviral factors are among the fastest evolving in the primate genome. The fact that the greatest polymorphism tends to be found in those parts of the gene that encode protein surfaces interacting directly with retroviral proteins, along with the fact that retroviruses encode proteins that specifically degrade many of these antiviral factors, supports the contention that the evolution of these antiviral genes is driven by retroviral challenge (1–3).

One such antiretroviral factor is called TRIM5, a member of the large tripartite motif family, of which humans have 100 genes (4). Like all TRIM family members, TRIM5 consists of a RING E3 ubiquitin ligase domain, a B box domain, and an extended, central, alpha-helical domain (5). The polymorphic C terminus comes as either a PRYSPRY or a cyclophilin domain, either of which constitutes the main species specificity determinant for CA recognition. The protein is believed to reside in the cell cytoplasm as a dimer where it meets the incoming retroviral CA lattice. If the CA lattice is recognized, TRIM5 forms a complementary lattice

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TABLE 1 Murine TRIM5 orthologues studied here

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Alternative gene names</th>
<th>Accession number</th>
<th>Protein domain(s)</th>
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<td>RBCC</td>
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</tbody>
</table>

(6) that promotes premature disassembly of the viral CA lattice (7), blocking reverse transcription, nuclear entry, and integration.

It appears, though, that CA recognition and TRIM5 lattice formation are not sufficient for full TRIM5 restriction activity. Several lines of evidence suggest that proteasomes contribute to the restriction mechanism. Proteasome inhibitors release reverse transcription from the TRIM5-mediated block, though a block to nuclear entry still remains (8). TRIM5 binds to proteasome components, and cytoplasmic bodies colocalize with proteasomes in cells after challenge with TRIM5-sensitive viruses (9, 10). These results suggest that at least some aspect of the restriction mechanism involves ubiquitination of the virion capsid with subsequent protein degradation in the proteasome. Additionally, TRIM5 has been reported to interact with cellular regulators of autophagy and to deliver restriction-sensitive retroviral capsids to autophagosomes for destruction (11, 12).

E3 ubiquitin ligase activity associated with the N-terminal RING domain also contributes to restriction activity (13). Though ubiquitinated retroviral capsid proteins have never been detected within cells in a TRIM5-dependent manner, it is still possible that the RING domain contributes to restriction activity by ubiquitinating the retroviral capsid. Alternatively, it has been reported that TRIM5 activates innate immune signaling (14–16) and that TRIM5 generates free K63-linked ubiquitin chains that activate the TAK1 (MAP3K7) kinase complex in vitro (15). Knockdown of TAK1, or of the specific E2 enzymes required for TRIM5 to generate K63-linked ubiquitin chains, attenuates human or owl monkey TRIM5-mediated restriction (15). These results suggest that TAK1-dependent innate immune signaling, activated by TRIM5 E3 ubiquitin ligase activity, may also contribute to the restriction mechanism.

TRIM5 retroviral restriction activity has been most extensively studied among primates (2), each of which has one TRIM5 gene, the products of which have restriction specificity for different subsets of retroviruses (17). An extraordinary expansion of the TRIM5 locus is found in rodents, such that rats have 3 TRIM5 genes and mice have at least seven (18). Given the panel of retroviral vectors that are currently available for phenotyping testing, none were found to be restricted by any of the rodent genes tested (18). This could mean that the rodent TRIM5 genes lack retroviral restriction activity or that a restriction-sensitive virus has just not been found.

Here, we tested 18 different TRIM proteins for the ability to activate innate immune signaling. The panel included seven murine Trim5 homologues (Table 1), which, in addition, were fused to cyclophilin A (CypA) in order to determine if they were capable of retroviral restriction.

MATERIALS AND METHODS

Drugs, reagents, and antibodies. The TAK1 inhibitor S6-7-oxo-aceanol (Sigma) was diluted in dimethyl sulfoxide (DMSO) and used at a concentration of 300 nM. Puromycin and hygromycin (Sigma) were used at 5 μg/ml and 50 μg/ml, respectively. Mouse anti-c-Myc and anti-β-actin antibodies were from Sigma. Rabbit anti-TAK1 and anti-β-tubulin antibodies were from Cell Signaling Technologies.

Cell lines. HEK293, CRFK, and C57BL/6 mouse embryonic fibroblast (MEF) cell lines were maintained in high-glucose Dulbecco’s modified Eagle’s medium (D-MEM) (Invitrogen) supplemented with 1-glutamine (2 mM), penicillin (100 units/ml), streptomycin (100 μg/ml) (Gibco), and fetal bovine serum (FBS) (10%) (PA Laboratories).

Cloning and plasmids. The sequences of primers used for cloning are shown in Tables S1 and S2 in the supplemental material. Human and nonhuman primate TRIM5 orthologues were PCR amplified from previously described plasmid templates (15, 19–21). Feline TRIM5 was amplified by reverse transcriptase PCR (RT-PCR) from CRFK cell RNA. Human TRIM5 paralogues were amplified from human macrophage RNA. Murine Trim5 orthologues were amplified from C57BL/6 mouse embryonic fibroblast or splenic T cell RNA. Murine Trim5 orthologue fusions to cyclophilin A were generated by overlapping PCR using each mouse Trim and the cyclophilin A domain from owl monkey, TRIM5Cyp (20), as the templates.

Using standard methods, TRIM5 homologues and fusion proteins were cloned into expression plasmid pcDNA3.I(−) (Invitrogen) or into pFUPI, which encodes an human immunodeficiency virus type 1 (HIV-1)-based, lentiviral genome with an internal ubiquitin promoter driving expression of a puromycin resistance cassette followed by the encephalomyocarditis virus (ECMV) internal ribosome entry site (IRES) (22). The coding sequences of interest were all cloned 3′ of the IRES.

pWP7Ts-GFP is an HIV-1-based transfer vector with enhanced green fluorescent protein (EGFP) expression under the control of the EF1α promoter; psPAX2 and pMD2.G encode HIV-1 gag-pol and the vesicular stomatitis virus glycoprotein (VSV-G), respectively (23). HIV-1-GFP has a frameshift mutation in Env, and Nef is replaced with GFP (24). pLNCx-GFP and pCCG-gagpol are Moloney murine leukemia virus (MLV) genome and gag-pol plasmids, respectively. The Prl promoter AP-1-luc construct was a gift from Ruslan Medzhitov (Yale School of Medicine).

Transfection and vector production. FUPI three-part virus was obtained by transfecting 293FT cells, plated in 10-cm plates, with pFUPI plasmid containing either no insert or different TRIM-Cyp constructs or psPAX2 (gagpol) and pMD2.G encoding HIV-1-GFP has a frameshift mutation in Env, and Nef is replaced with GFP (24). pLNCx-GFP and pCCG-gagpol are Moloney murine leukemia virus (MLV) genome and gag-pol plasmids, respectively. The Prl promoter AP-1-luc construct was a gift from Ruslan Medzhitov (Yale School of Medicine).

Transfection assays. CRFK cells expressing Trim-CypA fusions or empty vector were seeded at a density of 1.5 × 10⁴ cells/well in a 96-well plate. HEK293 cells stably coexpressing Trim-CypA fusions or NT or TAK1 shRNAmir were seeded at a density of 2 × 10⁴ cells/well in a 96-well plate. Twenty-four hours later, the cells were infected with serial dilutions of HIV-1-GFP three-part vectors were prepared by transfecting HEK293 cells in 10-cm plates with pWPTs-GFP, psPAX2, and pMD2.G (envelope) plasmids (22). HIV-1-GFP three-part vectors were prepared by transfecting HEK293 cells in 10-cm plates with pWPTs-GFP, psPAX2, and pMD2.G at a ratio of 3:2:1, using Lipofectamine 2000 (Invitrogen) or polyethyleneimine (PEI) (Sigma). Thirty micrograms of DNA was mixed with 60 μg/ml of Lipofectamine 2000 or PEI (1 mg/ml) in 1 ml of Opti-MEM (Invitrogen), incubated for 30 min, and added to the cells. At 48 h posttransfection, virus supernatants were harvested.

Luciferase transcription reporter assays. HEK293 cells were seeded on white 96-well plates (Perkin-Elmer) at a density of 2.5 × 10⁴ cells per well. Twenty-four hours later, cells were transfected with 25 ng of firefly luciferase reporter plasmid, 5 ng of the internal control reporter plasmid pRL-TK (Promega), and 25 to 50 ng of the pcDNA3.1(−) plasmid con-
was measured and normalized to renilla luciferase in each sample. Bars show the means with anti-Myc or anti-actin antibodies.

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Activation of innate immune signaling is a conserved feature of TRIM5 orthologues from primates, Carnivora, and Rodentia. To determine if the activation of innate immune signaling is a conserved property among TRIM5 family members, TRIM5alpha from Homo sapiens, Macaca mulatta, Ceropithicus aethiops, and Ceropithicus tantalus; TRIM5-cyclophilin A fusions from Aotus trivirgatus and Macaca nemestrina; and TRIM5 from Felis catus were overexpressed by plasmid transfection of HEK293 cells. In each case, an AP-1 luciferase reporter was activated, with the magnitude ranging from 25-fold (Ceropithicus aethiops) to 262-fold (Aotus trivirgatus) above the levels with empty expression plasmid (Fig. 1A). In contrast, the paralogues most closely related to human TRIM5alpha either had undetectable (TRIM6 and TRIM22) or minimal (TRIM34) activity (Fig. 1B). The more distantly related human TRIM25, a protein required for RIG-I-mediated signaling (25), was nearly as potent as TRIM5alpha (Fig. 1B).

The Trim5 locus in rodents expanded by successive duplications such that the C57BL/6J mouse strain possesses seven Trim5 gene homologues (Table 1), Trim12a, Trim12b, Trim12c, Trim30a, Trim30b, and Trim30c (18). Coding sequence for each of these genes was cloned into an expression vector. When transfected into HEK293 cells, Trim12a, Trim12b, and Trim12c activated AP-1 as well as human TRIM5alpha (Fig. 1B). In contrast, none of the Trim30 genes caused significant induction of AP-1 (Fig. 1C). Trim12b, Trim12c, Trim30a, Trim30c, and Trim30d each are predicted to encode a full tripartite motif protein with the PRYSPRY domain typical of the TRIM5alpha isofrom (Table 1). Trim12a and Trim30b encode the core tripartite motif protein without the PRYSPRY domain. In each case, the gel mobility of the encoded proteins was as expected (Fig. 1D). Though steady-state levels of Trim30c protein were relatively low, the levels of Trim30a, Trim30b, and Trim30d were all higher than the levels of the Trim12 proteins (Fig. 1D). Thus, steady-state protein levels did not account for the reduced ability of the Trim30 gene to stimulate AP-1.
HEK293 cells were transfected with expression plasmids encoding each of the murine Trim proteins with a C-terminal Myc epitope. The transfected cells were probed by indirect immunofluorescence and visualized by confocal microscopy. Trim12a, Trim12b, and Trim12c all formed distinct cytoplasmic bodies (Fig. 2). In contrast, though some cytoplasmic bodies were observed with Trim30a, for the most part relatively diffuse cytoplasmic staining was observed with the Trim30 proteins (Fig. 2). These results indicate that all Trim5 homologues that activate innate immune signaling form discrete cytoplasmic bodies.

Retroviral restriction activity correlates with the ability to activate signaling. In an attempt to detect retroviral restriction activity associated with the mouse Trim5 homologues, CRFK cell lines that stably expressed each of them were generated, using previously described methods (19). These lines were then challenged with single-cycle vectors for HIV-1, HIV-2, the MAC strain of simian immunodeficiency virus (SIV<sub>MAC</sub>), equine infectious anemia virus (EIAV), and Moloney murine leukemia virus. In agreement with previous attempts by others (18), no restriction activity was detected with any of the viruses. However, the strong signature of positive selection reported for the rodent Trim5 homologues (18) suggests that, as demonstrated with the primate TRIM5 orthologues (2, 17), the rodent genes have also been selected to protect against retroviral infection and that the viruses restricted by them remain to be identified.

TRIM5-mediated retroviral restriction activity depends upon multiple biochemical functions provided by independently acting modular domains within the protein (5). The RING domain exhibits E3 ubiquitin ligase activity. The central antiparallel, alpha-helical stalk (coiled-coil domain), along with the B-box domain,
dimerizes and multimerizes TRIM5. The polymorphic C terminus, either a PRYSPRY or cyclophilin A domain, binds directly to the retroviral CA lattice and therefore is the main CA specificity determinant. Lack of retroviral restriction activity could simply result from inability of a given TRIM5 protein to bind to the CA lattice of the particular virus under investigation. To determine if this was the case, the RING, the B box, and the coiled-coil domains (RBCC) of the murine Trim5 homologues were fused to the well-characterized HIV-1 CA binding protein cyclophilin A (CypA), as has been done with other TRIM proteins (22, 26). As a control, CypA-H126Q was used; this mutant protein does not bind to the HIV-1 CA (27).

When transfected into HEK293 cells, Trim12a-CypA and Trim12a-CypA-H126Q each induced the AP-1 reporter about 100-fold, similar to Trim12a without the fusion (Fig. 3A). In contrast, Trim30a had minimal effect on the reporter, and when fused to CypA, there was minimal increase in activity over the unfused protein (Fig. 3A). Trim12b induced the AP-1 reporter 50-fold, and the Trim12b-CypA fusion protein induced it 200-fold (Fig. 3B). Trim12c and Trim12c-CypA each induced AP-1...
over 150-fold (Fig. 3B). Trim30b and Trim30b-CypA had minimal effect on AP-1 activity (Fig. 3B). While the CypA fusion caused some increase in AP-1 activity for Trim12b and Trim30a, in general the effect of the CypA fusions on signaling was similar to that of the unfused proteins.

To determine if the CypA fusion proteins were able to restrict HIV-1, a lentiviral expression vector (Fig. 3C) was used to transduce highly permissive CRFK cells. This bicistronic vector confers puromycin resistance, and transduced cells were selected in pools. These were then challenged with a three-part, vesicular stomatitis virus G protein (VSV-G)-pseudotyped, HIV-1 vector bearing a GFP reporter genome. Cells expressing the Trim12a-CypA fusion gene were about 100-fold less permissive for transduction than were the cells bearing either empty vector, Trim12a, or Trim12a fused to the CypA-H126Q mutant (Fig. 3D). In contrast, Trim30a or Trim30a fused to CypA had no detectable restriction activity (Fig. 3D). Trim12b-CypA and Trim12c-CypA each had restriction comparable to that of Trim12a-CypA, though neither unfused protein nor Trim30b-CypA had clear restriction activity (Fig. 3E). Steady-state protein levels for the CypA protein fusions did not explain the inability of the Trim30-CypA fusion proteins to restrict HIV-1, in that protein levels for Trim30a-CypA and Trim30b-CypA were much higher than were those for the Trim12 fusion proteins. Restriction activity of Trim12A-CypA was not due to nonspecific activation of an antiviral state, in that this protein had no effect on transduction by Moloney murine leukemia virus (Fig. 3F); the capsid of this virus is not recognized by CypA (28). Taken together, these results indicate that restriction activity requires CA recognition and the ability to stimulate AP-1 luciferase activity.

**TAK1** is required for activation of the mitogen-activated protein kinase (MAPK) pathway by mouse TRIM5 orthologues. Activation of innate immune signaling by human TRIM5 can be prevented by 5Z-7-oxozeaenol, a specific inhibitor of transforming growth factor β (TGF-β)-activated kinase 1 (TAK1 or MAP3K7) (15). To determine if TAK1 is required for activation of innate immune signaling by the murine Trim5 homologues, HEK293 cells were transfected with expression plasmids for the Trim12 genes in the presence of 5Z-7-oxozeaenol. AP-1 luciferase activity associated with Trim12a was decreased 5-fold by the kinase inhibitor (Fig. 4A). With either Trim12b or Trim12c, the kinase inhibitor decreased AP-1 activity 11-fold (Fig. 4A).

Genetic evidence for the importance of TAK1 was obtained by stable transduction of HEK293 cells using a previously described lentiviral vector that expresses a puromycin resistance gene and miR30 modified to knock down TAK1 (15, 16). A vector targeting irrelevant sequences was used as a control. The two pools of puromycin-resistant cells were transfected with increasing amounts of the Trim12 expression plasmids. TAK1 knockdown (KD) reduced AP-1 induction by Trim12a 20-fold and induction by Trim12c 25-fold (Fig. 4B). The combination of TAK1 knockdown with overexpression of Trim12b proved too toxic to permit meaningful analysis of the results. Taken together, these results indicate that the murine TRIM5 orthologues stimulate innate immune signaling through TAK1.

**TAK1** is required for HIV-1 restriction by murine Trim12-cyclophilin A fusion proteins. To determine if HIV-1 restriction by murine Trim12-cyclophilin A fusion proteins requires innate immune signaling, doubly transduced HEK293 cell lines were generated, using vectors as described previously (29). The first round of transduction was with hygromycin resistance-conferring, miR30 knockdown vectors targeting TAK1 or control, followed by transduction with puromycin resistance-confering vectors expressing the Trim12-CypA fusion genes. The pools of hygromycin- and puromycin-double-resistant cells transduced with Trim12b-CypA grew poorly and could not be assessed fur-
ther. The remaining pools of doubly resistant cell lines were then challenged with a VSV-G-pseudotyped, env-minus, HIV-GFP vector. Trim12A-CypA and Trim12C-CypA restricted HIV-1 transduction between 5- and 10-fold, compared with cells transduced with the empty puromycin resistance expression vector, in the presence of the control knockdown construct (Fig. 4C). In contrast, cells expressing the TAK1 shRNAmir did not inhibit HIV-1 transduction, indicating that TAK1 was required for Trim12a-CypA and Trim12c-CypA restriction activity.

DISCUSSION

Here, we tested 18 TRIM family members for the ability to activate innate immune signaling. Among these, six out of six primate TRIM5 orthologues had the ability to activate AP-1 signal transduction (Fig. 1A). The primate TRIM5 proteins were selected from both Old World and New World primates and included proteins that utilize a PRYSPRY domain or a CypA domain to recognize the incoming capsid lattice. Each of these TRIM5 proteins is capable of restricting retroviruses, though their capsid specificities are quite diverse. For example, owl monkey TRIM5-CypA and rhesus TRIM5alpha potently block HIV-1 infection (20, 30, 31), while rhesus TRIM5-CypA and human TRIM5alpha have minimal activity against this virus (21, 30, 32, 33). Instead, rhesus TRIM5-CypA potently restricts HIV-2 (21, 32) and human TRIM5alpha potently restricts N-tropic MLV or EIAV (34–37).

Among Carnivora, the TRIM5 gene in dogs was disrupted by insertion of a retrotransposed PNRC1 pseudogene (38). Cats have a TRIM5 gene, but it lacks coding sequence for the C-terminal CA binding domain (39). Nonetheless, when fused to the HIV-1 CA binding protein CypA, the feline orthologue has potent HIV-1 restriction activity (39). Feline TRIM5 was tested here and also found to activate AP-1 signaling (Fig. 1A). In contrast, the three closest human paralogues of TRIM5, TRIM6, TRIM22, and TRIM34, do not activate AP-1 signaling (Fig. 1B). Interestingly, TRIM25, a paralogue that is required for RIG-I-mediated signal transduction (25) and that generates K63-ubiquitin chains like TRIM5 (40), also activates AP-1 signaling.

Among the seven mouse Trim5 homologues tested here, Trim12a, -b, and -c all activated innate immune signaling, and the Trim30 proteins did not (Fig. 1C). An obvious question of interest is which residues in these proteins explain the differences in activity. To address this issue, a panel of chimeras was generated in which domains were swapped between Trim12a and Trim30a. These two proteins possess 60% amino acid identity (see Fig. S1 in the supplemental material). Unfortunately, the activity could not be pinpointed to specific amino acid residues or even to a specific domain. This kind of mapping has been difficult with TRIM5 proteins, as observed previously (12, 15).

According to the work of Tareen et al. and confirmed by us, none of the murine Trim5 homologues had restriction activity against any of eight different retroviral vectors (18). For Trim12a and Trim30b, absence of retroviral restriction activity is not surprising given that these genes encode only the core Trim5 RBCC isoform (RING, B box, and coiled-coil domain) and lack a C-terminal CA binding domain (Table 1). For the other homologues that possess a C-terminal PRYSPRY domain (Trim12b and -c and Trim30a, -c, and -d), lack of restriction activity could be because the encoded proteins actually lack restriction activity or simply because a restriction-sensitive retrovirus has not yet been identified. To bypass our ignorance regarding this question, the HIV-1 CA binding protein CypA was fused via flexible linker to the C terminus of the RBCC portion of each of the murine Trim5 homologues (Fig. 3C), as we and others have done previously (22, 26). Among the fusion proteins, only those Trim5 homologues that activated AP-1 signaling exhibited HIV-1 restriction activity. It is of note that those murine Trim5 homologues that activated innate immune signaling and that when fused to CypA had HIV-1-specific restriction activity formed discrete cytoplasmic bodies without any diffuse cytoplasmic signal (Fig. 2). This was not the case with the Trim30 proteins. Similar correlation of restriction activity with the ability to form only discrete cytoplasmic bodies was reported previously with a panel of engineered human TRIM5-CypA fusion proteins (22). Thus, the ability to activate innate immune signaling and to form discrete cytoplasmic bodies appears to be required for TRIM5-mediated retrovirus restriction.

Despite the fact that we and others were unable to identify a retrovirus that is restricted by the murine Trim5 homologues, signatures of positive selection have been reported in the mouse genes (18), suggesting that the selective pressure for Trim5 expansion derives from challenge with retroviruses, as is believed to be the case with primate TRIM5 (2). But why did the murine Trim5 locus expand so enormously? One obvious difference between mouse and human is that in humans there are no known replication-competent, endogenous retroviruses, whereas in mice there are many endogenous retroviruses with the ability to replicate and mutate the mouse genome (41).

Disruption of TAK1 either by a pharmacologic inhibitor or by knockdown prevented AP-1 activation by the murine Trim12 proteins (Fig. 4A and B). Similarly, TAK1 knockdown prevented the Trim12-CypA fusion proteins from restricting HIV-1 (Fig. 4C). TAK1 had been shown to coprecipitate with TRIM5 and to be required for the free K63 ubiquitin chains that TRIM5 synthesizes in the presence of the heterodimeric E2 enzymes UEV1A and UBC13 (15). These findings suggested that TRIM5 acts as an innate immune pattern recognition receptor specific for the capsid lattice. Recognition of antibodies that have been brought into the cytoplasm attached to viruses stimulates TRIM21 to synthesize ubiquitin machinery (47). Another possibility is that Tak1 regulates Trim5 stability via effects on ubiquitin machinery (47).
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