Trim5α Accelerates Degradation of Cytosolic Capsid Associated with Productive HIV-1 Entry*

Udayan Chatterji, Michael D. Bobardt, Peter Gaskill, Dennis Sheeter, Howard Fox, and Philippe A. Gallay

The TRIM5α (tripartite motif 5α protein) has been linked to the cross-species restriction in human immunodeficiency virus type 1 (HIV-1) infection of non-human cells, but the mechanism by which this occurs remains to be fully elucidated. Here we demonstrate that the capsid (CA) protein of HIV-1 is more rapidly degraded in cells expressing monkey TRIM5α than in cells expressing human TRIM5α. Other proteins encoded by Gag and Pol are not subject to TRIM5α-mediated accelerated degradation. The accelerated CA degradation by TRIM5α apparently occurs via a nonproteosomal pathway. TRIM5α selectively accelerates degradation of the CA population, which reached the cytosol of restrictive cells, but not the CA population, which ended into the vesicular compartment. Given that cytosolic CA represents “productively” entered cores, whereas vesicular CA represents “nonproductively” entered cores, our findings suggest that TRIM5α interrupts the infectious pathway of HIV-1 by acting on the incoming cytosolic CA. The mode of viral entry does not influence the accelerated degradation of cytosolic CA by TRIM5α. Thus, this study reveals a correlation between TRIM5α-mediated HIV-1 restriction and a selective degradation of cytosolic CA normally associated with productive viral entry.

For successful infection, the HIV-12 CA core has to undergo a series of critical events following its delivery into the host cell. These events include the breakdown of the shell of CA that surrounds the viral RNA (uncoating), the conversion of the viral RNA into DNA (reverse transcription), migration of the viral DNA to the nucleus (nuclear import), and ultimately the insertion of the viral DNA into the host chromosomes (integration). Because HIV-1 encounters a block in monkey cells after entry but prior to integration, it has been speculated that monkeys contain factors that interfere with HIV-1 uncoating and/or reverse transcription (1–7). The first monkey restriction factor was identified, the Rhesus monkey (RhM) TRIM5α (8). Interfering with RhMTRIM5α expression in monkey cells abolishes HIV-1 restriction (8). Furthermore, expression of RhMTRIM5α in human cells, which are normally permissive to HIV-1, restricts HIV-1 (8). Humans (Hu) also express TRIM5α, which, although 87% identical in amino acid sequence to RhMTRIM5α, weakly restricts HIV-1 because of substitutions in the C terminus (8–11). Anti-HIV-1 restriction activities have been attributed to other monkey TRIM5 proteins such as African green and spider monkey TRIM5α proteins (12–15). Moreover, a unique form of TRIM5 exists in owl monkeys, TRIM5-CypA, which also restricts HIV-1 and which results from the retrotransposition of a cyclophilin A (CypA) pseudogene into the TRIM5 locus (16, 17). Thus, monkey TRIM5α and TRIM5-CypA proteins represent a novel class of intracellular innate immunity factors that arrest HIV-1 during the early steps of infection. Swapping HIV-1 CA for RhM CA renders HIV-1 incapable of infecting RhM cells (18). Moreover, CA mutations alter the sensitivity of HIV-1 to TRIM5α in monkey cells (19–24). This suggests that the CA encodes the determinants for TRIM5α restriction. However, although it is clearly established that TRIM5α and TRIM5-CypA block HIV-1 after viral entry but before integration (1, 8, 25–29), it is still poorly understood how these restriction factors interrupt the virus pathway. We thus investigated the mechanisms that control the block in HIV-1 infectivity by TRIM5α. Specifically, we asked whether monkey TRIM5α and TRIM5-CypA block HIV-1 infection by diverting the incoming CA core from the infective pathway into an abortive degradation pathway.

EXPERIMENTAL PROCEDURES

Viruses and Infections—The pNL4.3-GFP plasmid was provided by C. Aiken and D. Gabuzda. The G89V CA-GFP mutant was generated by PCR mutagenesis (24). The non-GFP-expressing wild-type, G89V pNL4.3, and VSVG plasmids were provided by D. Trono. NL4.3 Env minus plasmid was provided by C. Aiken. Viruses were generated by transfection of 293T cells using GeneJuice (EMD Biosciences, Inc., Novagen). Viral supernatants were harvested 48 h post-transfection and filtered through a 0.2-μm pore size filter. Viral inoculum was standardized by exoRT assay or p24 ELISA (PerkinElmer Life Sciences). HeLa-HuTRIM5 and HeLa-RhMTRIM5 were provided by J. Sodroski (8), and OMK cells were provided by G. Towers and C. Aiken. The pcDNA-CD4 plasmid provided by M. Goldsmith.

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1 To whom correspondence should be addressed: Dept. of Immunology IMM-9, The Scripps Research Institute, 10550 North Torrey Pines Rd., La Jolla, CA 92037. Tel.: 858-784-8180; Fax: 858-784-8227; E-mail: gallay@scripps.edu.

2 The abbreviations used are: HIV-1, human immunodeficiency virus, type 1; Hu, human; RhM, Rhesus macaque; VSVG, vesicular stomatitis virus G protein; OMK, Owl monkey kidney; CA, capsid; MA, matrix; NC, nucleocapsid; RTC, reverse transcription complex; CypA, cyclophilin A; ELISA, enzyme-linked immunosorbent assay; GFP, green fluorescent protein; FACS, fluorescence-activated cell sorter; PNF, post-nuclear fraction.
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was used to create stable CD4+ HeLa-HuTRIM5 and -RhMTRIM5 cell lines. Debio-025 was provided by DEBIOP-HARM. Debio-025 (2 μM) was added 15 min prior to virus addition. Infected cells were analyzed by FACS (GFP content) 48 h post-infection. For saturation experiments, OMK cells were initially exposed to increasing amounts of virus that does not encode GFP and subsequently (5 min later) exposed to wild-type HIV-1 that does encode GFP.

Degradation Analysis of Incoming HIV-1 Components in Infected Cells—Cells were exposed to HIV-1 (25, 100, and 250 ng of p24) for 1 h at 4°C to permit virus attachment and washed extensively to remove unbound virus to synchronize subsequent virus internalization. We synchronized viral internalization to avoid a continuous entry of viruses that would interfere with the interpretation of the degradation results. For each experiment, we verified that similar amounts of virus were initially attached onto the surface of target cells by Western blot and ELISA (data not shown). After washes to remove unattached virus, cells were placed at 37°C for subsequent viral internalization. After different time points, cells were trypsinized and lysed in 0.5% Nonidet P-40 for whole lysate isolation. Nuclear extracts were discarded by centrifugation. Whole lysates were standardized for protein concentration and analyzed for viral content by Western blot. Cytosolic and vesicular extracts from infected cells were isolated as described previously (30) with minor modifications. After Dounce homogenization (16 strokes, 2 ml B pestle), nuclei and cell debris were spun down at 3000 rpm for 10 min, and resulting post-nuclear fraction (PNF) was spun at 41,000 rpm at 4°C for 18 min in an SW41 Ti rotor. PNF supernatant corresponding to the “cytosolic fraction” was carefully collected, trichloroacetic acid-precipitated (20% trichloroacetic acid final) on ice for 30 min, and spun in a microcentrifuge at 14,000 rpm for 20 min. Pellet was washed twice with 200 μl of ice-cold acetone, dried at 95°C for 5–10 min, and resuspended in SDS loading buffer. PNF pellet corresponding to the “vesicular fraction” was directly resuspended in SDS loading buffer.

Anti-HIV-1 protein polyclonal antibodies were obtained from the AIDS Research and Reference Reagent Program, National Institutes of Health, and rabbit anti-CypA serum was obtained by immunization with recombinant human CypA protein (24). The anti-CA polyclonal antibody (HIV-1 SF2 p24 antiserum) recognizes wild-type and G89V capsids equally well (24). Real time PCR analysis was performed as described previously (31). Amplification efficiencies were validated and normalized against glyceraldehyde-3-phosphate dehydrogenase, and fold increases were calculated using the Comparative CT Method for quantitation. The proteasome inhibitors MG132 and lactacystin (EMD Biosciences, Inc., Calbiochem) were added to target cells 30 min before infection at 25 and 20 μM, respectively.

RESULTS

Accelerated Degradation of Incoming HIV-1 CA in TRIM5α+ Restrictive Cells—We first sought conditions where TRIM5α exhibits potent inhibitory effect on HIV-1 infection. To address this issue, we infected cells that highly restrict HIV-1 such as HeLa cells that express RhMTRIM5α (HeLa-RhMTRIM5) or that poorly restrict HIV-1 such as HeLa cells that express human TRIM5α (HeLa-HuTRIM5) (8) (Fig. 1A). The introduction of RhMTRIM5α in HeLa cells (HeLa-RhMTRIM5), but not HuTRIM5α (HeLa-HuTRIM5), impairs the capacity of HIV-1 to infect these cells (Fig. 1A). Note that HIV-1 infects successfully and similarly parental HeLa and HeLa-HuTRIM5 cells (data not shown), suggesting that HuTRIM5α fails to restrict HIV-1 and thus represents an ideal control for RhMTRIM5α. Even high doses of HIV-1 barely infect restrictive HeLa-RhMTRIM5 cells (Fig. 1A), suggesting that RhMTRIM5α, but not HuTRIM5α, is a potent restriction factor against HIV-1 and that HeLa-RhMTRIM5 cells are adequate to analyze the fate of incoming CA in restrictive cells. The nature of the viral envelope (gp120 or VSVG) does not influence the degree of RhMTRIM5α-mediated restriction (Fig. 1A).

To determine whether TRIM5α blocks HIV-1 infection by targeting the incoming CA core for degradation, we examined the degradation status of HIV-1 CA in whole lysate as well as of other viral components in both restrictive and nonrestrictive cells during the early steps of infection. HIV-1 matrix (MA), which is delivered in the cytoplasm of target cells during the early steps of infection, is slowly but significantly degraded over time in both nonrestrictive and restrictive cells (Fig. 1B). In nonrestrictive cells, CA also undergoes a slow but significant degradation (Fig. 1B). In contrast, in restrictive cells, CA undergoes an accelerated degradation (Fig. 1B). To quantitatively evaluate the degradation rate of CA in whole lysate, we measured amounts of CA by ELISA. Supporting our Western blot data (Fig. 1B), the CA degradation is accelerated in restrictive cells (Fig. 1C). We observed that CA degradation in whole lysate is constantly more obvious by Western blot analysis than by p24 ELISA. This is certainly because of the fact that ELISA can detect intact, partially, and even largely degraded CA (peptides), whereas Western blot mainly detects intact CA. We verified that similar amounts of whole lysates were analyzed by comparing levels of CypA (Fig. 1B). Note that the same blot was successively probed with antibodies directed against various viral and cellular proteins. Our observation that the equally abundant incoming HIV-1 protein, MA, undergoes only slow degradation in restrictive cells, whereas CA undergoes rapid degradation (Fig. 1, B and C), suggests that CA is preferentially targeted for degradation in restrictive cells. These findings reveal a correlation between RhMTRIM5α-mediated HIV-1 restriction and an accelerated degradation of incoming CA. Surprisingly, reverse transcriptase (Fig. 1B), nucleocapsid (data not shown), and viral RNA (Fig. 1D) undergo only a slow but significant degradation in whole lysates of both nonrestrictive and restrictive cells. These findings suggest that, although incoming CA is selectively targeted for degradation in restrictive cells, the integrity of the inner core components is preserved. We observed that CA undergoes a more dramatic degradation in restrictive cells when the viral inoculum is low (data not shown), probably because of the fact that when less CA enters the cell, less CA is present to saturate the restriction factor. On the other hand, the inoculum needs to be sufficiently high to detect CA within the target cell by Western blot. We thus had to use three virus concentrations for each experiment. It is also important to note that although we observed some differences in the degree of CA degradation between experiments, we always...
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observed a significant and reproducible difference in the threshold of CA degradation between restrictive and nonrestrictive cells, supporting the notion that TRIM5α-mediated restriction correlates with accelerated CA degradation.

TRIM5α Accelerates Degradation of the CA Population Delivered into the Cytosol of Target Cells That Is Normally Associated with Productive Viral Entry—We showed that incoming CA is more rapidly degraded in restrictive than in nonrestrictive cells. However, what we examined was the degradation status of CA in whole lysate and not the cytosolic CA population that has been shown to contain authentic infectious cores associated with productive entry ("productively" entered cores) (30). Thus, we examined whether TRIM5α-mediated restriction correlates with selective degradation of cytosolic CA. In both nonrestrictive and restrictive cells, CA accumulates in the vesicular compartment and then disappears (Fig. 2a). Our data indicate that the CA population, which ends into the vesicular compartment, undergoes degradation. This supports the notion that CA delivered into the vesicular compartment represents abortive infectious events ("nonproductively" entered cores) (30). Because a similar pattern of degradation of vesicular CA is observed in both nonrestrictive and restrictive cells (Fig. 2a), this suggests that TRIM5α does not exert its restriction effect by acting on vesicular CA. In sharp contrast, the CA population delivered into the cytosol of nonrestrictive cells remains perfectly intact even 4 h post-infection, whereas it undergoes degradation in restrictive cells (Fig. 2a). This indicates that RhMTRIM5α accelerates degradation of the cytosolic CA population, which represents the productively entered cores. The CA degradation rate quantified by p24 ELISA or by Western blot analysis correlates well (Fig. 2, right graphs). We examined the subcellular localization of TRIM5α and found it mainly cytosolic (Fig. 2d), suggesting that the restriction factor is well positioned to recognize incoming cores as soon as they are delivered into the cytosol. Because we found TRIM5α and CypA mainly cytosolic (not in the vesicular compartment), this demonstrates that our subcellular fractionation works properly. Our finding that RhMTRIM5α-mediated restriction correlates with degradation of cytosolic CA further supports the notion that cytosolic CA represents productively entered cores, whereas vesicular CA represents nonproductively entered cores (30). Our observation that vesicular CA is similarly degraded in restrictive and nonrestrictive cells, whereas cytosolic CA is more rapidly degraded in restrictive than in nonrestrictive cells, suggests that the TRIM5α-mediated accelerated CA degradation we observed above in whole extracts (Fig. 1) mainly arose from cytosolic CA degradation rather than vesicular CA degradation. Thus, the apparent "modest" effect of TRIM5α on CA degradation compared with the "dramatic" effect of TRIM5α on HIV-1 infection can be explained by the selective degradation of the subset of CA delivered into the cytosol of restrictive cells that represents productively entered cores.

The Mode of Viral Entry Does Not Influence Accelerated Cytosolic CA Degradation by TRIM5α—To date, we have exclusively used VSVG-pseudotyped viruses, and we asked whether the mode of entry influences the RhMTRIM5α-mediated CA degradation. We thus compared CA degradation in cells exposed to a virus that expresses VSVG or gp120. In the absence of envelope, no CA is detected in cytosolic extracts (Fig. 2b). Importantly, we observed a similar pattern of vesicular
and cytosolic CA degradation in restrictive cells infected with gp120-pseudotyped (Fig. 2c) or VSVG-pseudotyped viruses (Fig. 2a). Thus, the mode of entry does not affect TRIM5α-mediated CA degradation. This is in accordance with the fact that RhMTRIM5α restricts VSVG-pseudotyped or gp120-pseudotyped HIV-1 equally well (Fig. 1A).

TRIM5α-mediated Accelerated Degradation of Incoming HIV-1 CA Is Apparently Independent of the Proteasome—Because TRIM5α contains a ubiquitin ligase domain, it has been envisioned that TRIM5α interrupts HIV-1 infection by proteasome degradation of incoming viral proteins (32). However, inactivation of RhMTRIM5α ubiquitin ligase activity, either by RING domain removal or by RING cysteine mutations, only partially diminished its restriction activity (8, 33). To determine the contribution of the proteasome to the RhMTRIM5α-mediated CA degradation, we examined the fate of incoming CA in nonrestrictive and restrictive cells treated with or without proteasome inhibitors. We thus examined the effect of proteasome inhibitors on CA present in whole lysate as well as in cytosolic and vesicular extracts.

Proteasome inhibitors enhanced the endurance of all incoming viral proteins, including MA and CA (Fig. 3A), reverse tran-
FIGURE 3. The antiviral activity of the proteasome on incoming CA is apparently distinct from that of RhMTRIM5α. A, HeLa-HuTRIM5 or HeLa-RhMTRIM5 cells were incubated with VSVG-HIV-1 in the presence or absence of proteasome inhibitors. Degradation rate of incoming HIV-1 proteins in whole lysates of infected cells was examined by Western blot. B, degradation rate of internalized CA in whole lysates was quantified by p24 ELISA. Results are expressed in percentage of cytoplasmic CA by fixing the percentage of CA 0.5 h post-internalization into untreated cells at 100. C, HeLa-HuTRIM5 and HeLa-RhMTRIM5 cells were infected with VSVG-HIV-1 (5–100 ng of p24) in the presence or absence of proteasome inhibitors. Percentage of infected GFP-positive cells was measured by FACS. D, degradation rate of incoming HIV-1 proteins, CypA and TRIM5α, in cytosolic and vesicular extracts of infected cells was examined by Western blot. Results are typical of those obtained in two independent experiments.
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scriptase, and NC (data not shown) in whole lysates of both nonrestrictive and restrictive cells. Proteasome inhibitors enhance CA endurance in nonrestrictive and restrictive cells equally (Fig. 3B), and they significantly increase HIV-1 infection in nonrestrictive and restrictive cells equally (Fig. 3C). This is in accordance with previous studies that showed that proteasome inhibitors increase HIV-1 infection via unknown mechanisms (34–36). However, CA degradation in whole lysates remains more severe in restrictive cells than in untreated nonrestrictive cells even in the presence of proteasome inhibitors (Fig. 3, A and B). Specifically, by ELISA, 80% of incoming CA in whole lysates is degraded in restrictive cells 5 h post-infection, whereas only 40% of incoming CA is degraded in nonrestrictive cells (Fig. 3B). This suggests that incoming cores undergo an additional degradation that selectively targets CA in restrictive cells.

After examining the effect of proteasome inhibitors on incoming CA in whole lysate, we examined their effect on CA populations delivered into the cytosolic (productive entry) or vesicular (abortive entry) compartments. Proteasome inhibitors enhance both cytosolic and vesicular CA endurance in nonrestrictive and restrictive cells equally (Fig. 3D). Although proteasome inhibitors significantly enhance the endurance of cytosolic CA, they do not fully preserve cytosolic CA integrity in restrictive cells as we observed in whole lysates (Fig. 3A). As above (Fig. 2), cytosolic CA remains perfectly intact over time (even 5 h post-infection) in nonrestrictive cells but undergoes significant degradation in restrictive cells (Fig. 3D). We frequently observed that cytosolic CA accumulation is maximal 2–3 h after cells, pre-exposed with HIV-1 for 1 h at 4°C, were placed at 37°C for viral internalization (data not shown). Cytosolic MA does not undergo an accelerated degradation in restrictive cells (Fig. 3D), suggesting a selective TRIM5α-mediated effect on CA. Both vesicular MA and CA undergo similar degradation in nonrestrictive and restrictive cells in the presence or absence of inhibitors. Proteasome inhibitors weakly preserve the integrity of vesicular CA (in contrast to cytosolic CA) (Fig. 3D), suggesting that proteases other than proteasomal proteases, possibly lysosomal proteases, are responsible for CA degradation in the vesicular compartment. Proteasome inhibitors significantly enhance TRIM5α accumulation in the cytosolic compartment of both nonrestrictive and restrictive cells (Fig. 3D). Because TRIM5α is ubiquitinylated, it is likely that proteasomes regulate its cell turnover. Interestingly, although TRIM5α is normally mainly cytosolic, proteasome inhibitors mediate accumulation of TRIM5α in the vesicular compartment. This may originate either from a relocation of a subset of TRIM5α proteins from the cytosolic compartment into the vesicular compartment or from protection against a proteosomal attack in the vesicular compartment. Because proteasome inhibitors do not fully preserve the integrity of cytosolic CA in restrictive cells, our data suggest that cytosolic CA is subjected to two proteolytic attacks, one that is proteasome-mediated and another that is RHMTRIM5α-mediated but apparently proteasome-independent.

OMK TRIM5-CypA, Like RHMTRIM5α, Accelerates Degradation of Incoming HIV-1 CA—To determine whether the RHMTRIM5α-mediated accelerated CA degradation represents a common event in restrictive monkey cells, we asked whether another restriction factor, TRIM5-CypA (16, 17), prevents HIV-1 infection of OMK cells by also targeting CA for degradation. To test this hypothesis, we sought conditions, which prevent CA recognition by TRIM5-CypA and that restore the capacity of HIV-1 to infect restrictive OMK cells. Mutating the CypA-binding region of CA (G89V substitution), adding a cyclosporin A analog (Debio-025) that binds to the CypA moiety of TRIM5-CypA (Fig. 4A), or saturating endogenous TRIM5-CypA by an excess of virus (Fig. 4B) all alleviate the restriction and restore the capacity of HIV-1 to infect OMK cells (16, 17, 24, 37). We then asked whether TRIM5-CypA accelerates CA degradation in whole lysates of restrictive OMK cells, whether this degradation is prevented by disrupting CA-TRIM5-CypA interactions, and whether saturating OMK cells at high multiplicity of infection preserves CA. We found that incoming CA is more rapidly degraded in whole lysates of OMK cells than equally abundant MA (Fig. 4C). This selective CA degradation in OMK cells is reminiscent of the accelerated CA degradation observed in HeLa-RhMTRIM5 cells (Fig. 1B). Together, our findings reveal a correlation between the presence of RhMTRIM5α and OMK TRIM5-CypA, a block in HIV-1 infectivity, and a selective accelerated degradation of incoming CA. Importantly, Debio-025 enhances the integrity of incoming CA in whole lysates (Fig. 4, C and D). Specifically, CA is only moderately degraded 4 h post-infection in Debio-025-treated cells, whereas it is significantly degraded in untreated cells (Fig. 4, C and D). In contrast to wild-type capsid, the G89V CA remains intact (Fig. 4, C and D). The endurance of G89V CA toward TRIM5-CypA-mediated degradation correlates with the capacity of G89V HIV-1 to infect OMK cells (Fig. 4A). Our findings that wild-type, but not G89V CA, undergoes more rapid degradation demonstrate the specificity of the TRIM5-CypA-mediated accelerated CA degradation. G89V CA remains intact even 4 h post-infection, whereas wild-type CA in the presence of Debio-025 starts to undergo degradation 4 h post-infection (Fig. 4, C and D). This correlates with our observation that Debio-025 partially restores HIV-1 infectivity in OMK cells (25%) compared with G89V HIV-1 infectivity (55%) (Fig. 4A). Furthermore, incoming CA is protected from degradation when OMK cells are infected with large amounts of virus (10 µg of p24) rather than physiological amounts of virus (10–100 ng of p24) (Fig. 4C). This is in accordance with our data above that show that pre-exposing OMK cells with high concentrations of virus restores the capacity of HIV-1 to infect OMK cells (Fig. 4B). Importantly, both pharmacological (Debio-025) and genetic (G89V mutation) interventions that prevent HIV-1 CA recognition by TRIM5-CypA protect CA from degradation and rescue HIV-1 infectivity in OMK cells. Thus, by using two distinct anti-HIV-1 restriction entities, RHMTRIM5α and OMK TRIM5-CypA, our data suggest that there is a relationship between TRIM5-mediated HIV-1 restriction and a selective accelerated degradation of incoming CA.

DISCUSSION

Although it is well established that monkey TRIM5α and TRIM5-CypA proteins block HIV-1 infection, it is still obscure how they interrupt the virus pathway. Immediately after entry
into restrictive cells, we found that incoming HIV-1 CA undergoes an accelerated degradation. The degradation is apparently specific because other equally abundant structural viral proteins, such as MA and NC as well as viral components that reside within the CA core (reverse transcriptase and viral RNA), do not undergo the TRIM5α/H9251-mediated accelerated degradation in restrictive cells.

We found that all incoming viral proteins (CA, MA, NC, and reverse transcriptase) are equally susceptible to proteasome degradation and that proteasome inhibitors enhance HIV-1 infection in both nonrestrictive and restrictive cells. Thus, degradation of incoming viral proteins by the proteasome may represent an intracellular defense against HIV-1 infection in both nonrestrictive and restrictive cells. Thus, degradation of incoming viral proteins by the proteasome may represent an intracellular defense against HIV-1 infection in both nonrestrictive and restrictive cells. Thus, degradation of incoming viral proteins by the proteasome may represent an intracellular defense against HIV-1 infection in both nonrestrictive and restrictive cells. Thus, degradation of incoming viral proteins by the proteasome may represent an intracellular defense against HIV-1 infection in both nonrestrictive and restrictive cells.

A central finding of the study is that in contrast to the proteasome-mediated proteolytic attack that degrades all incoming viral proteins (CA, MA, NC and reverse transcriptase), the TRIM5α-mediated degradation targets cytosolic (not vesicular) CA specifically (not G89V capsid) and apparently exclusively (not MA, NC, reverse transcriptase and viral RNA). The joint action of the “nonselective” degradation of all incoming viral proteins by the proteasome and the “selective” CA degradation by TRIM5α may contribute to an accelerated degradation of CA and therefore to the inability of HIV-1 to infect restrictive cells. Our data further suggest that the CA population that reaches the cytosolic compartment and that contains

![FIGURE 4. Conditions that prevent HIV-1 CA recognition by TRIM5-CypA protect incoming CA from accelerated degradation and rescue HIV-1 infectivity. A, OMK cells were incubated with increasing amounts of wild-type, wild-type in the presence of the cyclosporin A analog Debio-025, and of G89V CA mutant HIV-1 expressing GFP. Percentage of infected GFP-positive cells was measured by FACS. B, OMK cells were initially challenged with large amounts of non-GFP-encoding HIV-1 and subsequently exposed to wild-type HIV-1 expressing GFP. Percentage of infected GFP-positive cells was measured by FACS. C, degradation rate of incoming HIV-1 proteins in whole lysates of OMK-infected cells was examined by Western blot analysis. D, degradation rate of internalized HIV-1 CA was quantified by p24 ELISA in whole lysates. Results are expressed in percentage of CA in whole lysates by fixing the percentage of CA 0.5 h post-internalization at 100. Results are typical of those obtained in three independent experiments.]

TRIM5α-mediated degradation does not utilize the proteasome machinery to accelerate the degradation of cytosolic CA. Future studies should determine which cellular proteases are utilized by TRIM5α to accelerate cytosolic CA degradation. Although one cannot totally exclude the possibility that proteasome inhibitors disrupt the degradation of proteins by lysosomes and other endocytic pathways as recently reported (38), our data suggest that incoming CA is subjected to two distinct degradations in restrictive cells, a proteasome- and a TRIM5α-mediated degradation that is apparently proteasome-independent.

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productively entered cores represents a point of vulnerability in the HIV-1 life cycle that could be exploited.

During the course of this study, two independent groups reported findings, which shed light on the mechanisms of action of TRIM5α on HIV-1. In one study, Stremlau et al. (39) elegantly demonstrated that TRIM5α binding to CA (recombinant CA-NC proteins) represents a precondition for HIV-1 restriction. More importantly, they found that the presence of TRIM5α in target cells correlates with a decrease in the amount of particulate cytosolic HIV-1 as well as N-MLV CA. Interestingly, the loss of particulate N-MLV CA (but not of HIV-1 CA) was accompanied by an increase in soluble CA, presumably uncoated CA molecules (39). Based on the interesting findings, the authors proposed that TRIM5α restricts retroviral infection by specifically recognizing CA cores and promoting their premature disassembly (39). In another study, Wu et al. (40) showed that the reversible proteasome inhibitor MG132 enhances reverse transcription in both TRIM5α- nonrestrictive and TRIM5α+ restrictive cells. Although MG132 restores generation of HIV-1 late reverse transcription products in TRIM5α+ restrictive cells, HIV-1 infection and generation of 1- and 2-long terminal repeat circular reverse transcription products remain impaired (40). This is in accordance with our observation (Fig. 3C) as well as by others (41, 42) that proteasome inhibitors do not rescue HIV-1 infectivity in restrictive cells. The authors proposed that TRIM5α restricts HIV-1 in two distinct phases, altering the normal transport of the RTC to the nucleus and targeting the RTC to be degraded by the proteasome (40). Based on this model, the authors speculated that MG132, by blocking the second restriction phase, allows accumulation of nonfunctional full-length reverse transcription products (40). How could we reconcile these data with our present data, which show a correlation between TRIM5α-mediated restriction and selective degradation of cytosolic HIV-1 CA?

Stremlau et al. (39) were unable to show that the loss of HIV-1 CA particulates was accompanied by an increase of free CA. This may suggest that either uncoated HIV-1 CA molecules are too rapidly degraded to be detected as free CA molecules, that HIV-1 CA cores are degraded without uncoating, or that HIV-1 CA cores are directed into compartments distinct from the cytosolic compartment such as the vesicular compartment, where they eventually are uncoated and/or degraded. Thus, the loss of HIV-1 CA particulates in restrictive cells observed by Stremlau et al. (39) does not conflict with our results that showed that the presence of RhMTRIM5α correlates with a significant loss of cytosolic CA.

Several scenarios may explain how TRIM5α mediates both CA core uncoating and degradation. In one scenario, TRIM5α binds cores and directs them to compartments where they are uncoated and/or degraded (i.e. lysosomes). In another, TRIM5α permits the docking onto the core of proteases, which take the CA shell apart such that the inner RTC is no longer functional. In another, TRIM5α provokes core uncoating, and uncoated CA molecules are more susceptible to degradation than core-associated CA molecules. It is important to note that in contrast to our study, Stremlau et al. (39) did not use a synchronized system for virus entry. The absolute rates of core uncoating or CA degradation were thus not examined. This may also explain why surprisingly no CA core uncoating was observed in nonrestrictive cells, even 16 h post-infection (39).

It is important to emphasize that an accelerated CA degradation in restrictive cells can be detected only when the restriction is maximal (i.e. in HeLa-RhMTRIM5 or OMK cells, see Figs. 1A and 4). If target cells restrict HIV-1 only partially (i.e. 50% inhibition of infectivity), we were unable to observe a significant and reproducible difference in CA degradation between nonrestrictive and restrictive cells (data not shown). This suggests that either TRIM5α-mediated CA degradation can only be experimentally observed when the restriction is maximal or that TRIM5α blocks HIV-1 infection by interfering with more than one step in the retrovirus life cycle as recently suggested by Yap et al. (43). It is also important to note that the same highly restrictive HeLa-RhMTRIM5 cells were used to demonstrate CA core uncoating (39) and CA degradation (in this study).

Wu et al. (40) showed that proteasome inhibitors allow accumulation of nonfunctional full-length reverse transcription products in restrictive cells, whereas we showed that proteasome inhibitors do not fully preserve the integrity of incoming CA in restrictive cells. Several scenarios may explain how proteasome inhibitors restore reverse transcription in restrictive cells but fail to preserve full CA integrity and to restore infectivity. In one scenario, proteasome inhibitors, by blocking the second TRIM5α-mediated restriction phase, allow accumulation of integration-competent but nonfunctional reverse transcription complexes as suggested by Wu et al. (40). In another scenario, proteasome inhibitors, by preserving the integrity of reverse transcriptase, enhance the generation of full-length reverse transcription products in abortive compartments. We showed that proteasome inhibitors enhance the endurance of all incoming viral proteins, including CA, MA, NC, and reverse transcriptase. Thus, one can envision that proteasome inhibitors, by preserving the integrity of reverse transcriptase of cores trapped into an abortive compartment, allow accumulation of nonfunctional, full-length reverse transcription products (40). Given that complete reverse transcription occurs normally in nonrestrictive cells, this certainly explains the small but significant enhancement of accumulation of full-length reverse transcription products observed in nonrestrictive cells compared with the more profound enhancement observed in restrictive cells (40). In another scenario, proteasome inhibitors, by influencing the trafficking and/or turnover of TRIM5α, divert the productively entered cores from the infectious pathway into an abortive pathway. Proteasome inhibitors alter both self-ubiquitination and subcellular localization of TRIM5α (40–42). One can thus envision that altering physiological ubiquitination and/or localization of TRIM5α would allow the trapping of core-TRIM5α complexes into abortive compartments. Supporting this hypothesis, we showed that proteasome inhibitors significantly protect TRIM5α from degradation in infected cells and that they redirect a subset of TRIM5α proteins into the vesicular compartment (Fig. 3D). Thus, further work is required to determine the contribution of the proteasome in the TRIM5α-mediated restriction.

Future studies are necessary to determine whether TRIM5α-mediated CA degradation is truly the cause or simply the result of the TRIM5α-mediated restriction. Moreover, further work is
necessary to determine whether CA degradation and CA core uncoating are distinct or linked events, and if they are linked, which one is the consequence of the other, and most importantly, whether the two events are required to interrupt the infectious pathway of HIV-1.

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REFERENCES

1. Shibata, R., Sakai, H., Kawamura, M., Tokunaga, K., and Adachi, A. (1995) J. Gen. Virol. 76, 2723–2730
2. Himathongkham, S., and Luciw, P. A. (1996) Virology 219, 485–488
3. Hofmann, W., Schubert, D., LaBonte, J., Munson, L., Gibson, S., Scamarrass, J., Ferringo, P., and Sodroski, J. (1999) J. Virol. 73, 10020–10028
4. Stoye, J. P. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 11549–11551
5. Goff, S. P. (2004) Mol. Cell 16, 849–859
6. Bieniasz, P. D. (2004) Nat. Immun. 5, 1109–1115
7. Towers, G. J. (2005) Hum. Gene Ther. 16, 1125–1132
8. Stremlau, M., Owens, C. M., Perron, M. J., Kiessling, M., Auttissier, P., and Sodroski, J. (2004) Nature 427, 848–853
9. Yap, M. W., Nisole, S., and Stoye, J. P. (2005) Curr. Biol. 15, 73–78
10. Perez-Caballero, D., Hatziioannou, T., Yang, A., Cowan, S., and Bieniasz, P. D. (2005) J. Virol. 79, 8969–8978
11. Song, B., Gold, B., O’Huigin, C., Javanbakht, H., Li, X., Stremlau, M., Winkler, C., Dean, M., and Sodroski, J. (2005) J. Virol. 79, 6111–6121
12. Hatziioannou, T., Perez-Caballero, D., Yang, A., Cowan, S., and Bieniasz, P. D. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 10774–10779
13. Keckesova, Z., Yilen, I. M., and Towers, G. J. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 10780–10785
14. Yap, M. W., Nisole, S., Lynch, C., and Stoye, J. P. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 10786–10791
15. Song, B., Javanbakht, H., Perron, M., Park, D. H., Stremlau, M., and Sodroski, J. (2005) J. Virol. 79, 3930–3937
16. Sayah, D. M., Sokolskaja, E., Berthoux, L., and Luban, J. (2004) Nature 430, 569–573
17. Nisole, S., Lynch, C., Stoye, J. P., and Yap, M. W. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 13324–13328
18. Owens, C. M., Yang, P. C., Gottlinger, H., and Sodroski, J. (2003) J. Virol. 77, 726–731
19. Kootstra, N. A., Munk, C., Tonnu, N., Landau, N. R., and Verma, I. M. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 1298–1303
20. Ikeda, Y., Yilnen, L. M., Kahar-Bador, M., and Towers, G. J. (2004) J. Virol. 78, 11816–11822
21. Owens, C. M., Song, B., Perron, M. J., Yang, P. C., Stremlau, M., and Sodroski, J. (2004) J. Virol. 78, 5423–5437
22. Hatziioannou, T., Cowan, S., Von Schwedler, U. K., Sundquist, W. I., and Bieniasz, P. D. (2004) J. Virol. 78, 6005–6012
23. Yilen, L. M., Keckesova, Z., Wilson, S. J., Ranasinghe, S., and Towers, G. J. (2005) J. Virol. 79, 11580–11587
24. Chatterji, U., Bobardt, M. D., Stanfield, R., Ptak, R. G., Pallansch, L. A., Ward, P. A., Jones, M. J., Stoddart, C. A., Scalfaro, P., Dumont, J. M., Besseghir, K., Rosenwirth, B., and Gallay, P. A. (2005) J. Biol. Chem. 280, 40293–40300
25. Towers, G. J., Hatziioannou, T., Cowan, S., Goff, S. P., Luban, J., and Bieniasz, P. D. (2003) Nat. Med. 9, 1138–1143
26. Bieniasz, P. D. (2005) Proc. Natl. Acad. Sci. U. S. A. 101, 13324–13328
27. Cowan, S., Hatziioannou, T., Cunningham, T., Muesing, M. A., Gottlinger, H. G., and Bieniasz, P. D. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 11914–11919
28. Munk, C., Brandt, S. M., Lucero, G., and Landau, N. R. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 13843–13848
29. Hatziioannou, T., Cowan, S., Goff, S. P., Bieniasz, P. D., and Towers, G. J. (2003) EMBO J. 22, 385–394
30. Marechal, V., Clavel, F., Heard, J. M., and Schwartz, O. (1998) J. Virol. 72, 2208–2212
31. Corbell, J., Sheeter, D., Genini, D., Rought, S., Leoni, L., Du, P., Ferguson, M., Mays, D. R., Welsh, J. B., Fink, J. L., Sasik, R., Huang, D., Drenkow, J., Richman, D. D., and Gingeras, T. (2001) Genome Res. 11, 1198–1204
32. Joazeiro, C. A., and Weissman, A. M. (2000) Cell 102, 594–595
33. Javanbakht, H., Diaz-Griffero, F., Stremlau, M., Si, Z., and Sodroski, J. (2005) J. Biol. Chem. 280, 26933–26940
34. Schwartz, O., Marechal, V., Friguet, B., Arenzana-Seisdedos, F., and Heard, J. M. (1998) J. Virol. 72, 3845–3859
35. Frederickson, B. L., Wei, B. L., Yao, J., Luo, T., and Garcia, J. V. (2002) J. Virol. 76, 11440–11446
36. Wei, B. L., Denton, P. W., O’Neill, E., Luo, T., Foster, J. L., and Garcia, J. V. (2005) J. Virol. 79, 5705–5712
37. Forshey, B. M., Shi, J., and Aiken, C. (2005) J. Virol. 79, 869–875
38. Yu, G. Y., and Lai, M. M. (2005) J. Virol. 79, 644–648
39. Stremlau, M., Perron, M., Lee, M., Li, Y., Song, B., Javanbakht, H., Diaz-Griffero, F., Anderson, D. J., Sundquist, W. I., and Sodroski, J. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 5514–5519
40. Wu, X., Anderson, J. L., Campbell, E. M., Joseph, A. M., and Hope, T. J. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 7465–7470
41. Perez-Caballero, D., Hatziioannou, T., Zhang, F., Cowan, S., and Bieniasz, P. D. (2005) J. Virol. 79, 15567–15572
42. Diaz-Griffero, F., Li, X., Javanbakht, H., Song, B., Welikala, S., Stremlau, M., and Sodroski, J. (2006) Virology 349, 300–315
43. Yap, M. W., Mark, P. D., and Stoye, P. S. (2006) J. Virol. 80, 4061–4067