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**Capsid Proteins from Human Immunodeficiency Virus Type 1 and Simian  
Immunodeficiency Virus SIV<sub>mac</sub> can Coassemble into Mature Cores of Infectious  
Viruses**

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## ABSTRACT

We have recently shown that the Gag polyproteins from human immunodeficiency virus type 1 (HIV-1) and HIV-2 can coassemble and functionally complement each other. During virion maturation, the Gag polyproteins undergo proteolytic cleavage to release mature proteins including capsid (CA), which refolds and forms the outer shell of a cone-shaped mature core. Less than half of the CA proteins present within HIV-1 virion are required to form the mature core. Therefore, it is unclear whether the mature core in virions containing both HIV-1 and HIV-2 Gag consists of CA proteins from a single virus or from both viruses. To determine whether CA proteins from two different viruses can coassemble into mature cores of infectious viruses, we exploited the specificity of the tripartite motif 5 $\alpha$  protein from rhesus monkey (rhTRIM5 $\alpha$ ) to cores containing HIV-1 CA (hCA) but not the simian immunodeficiency virus SIV<sub>mac</sub> CA protein (sCA). If hCA and sCA cannot coassemble into the same core, when equal amounts of sCA and hCA are coexpressed, the infectivities of such virus preparations in cells should be inhibited less than twofold by rhTRIM5 $\alpha$ . However, if hCA and sCA can coassemble into the same core structure to form a mixed core, rhTRIM5 $\alpha$  would be able to recognize such cores and significantly restrict virus infectivity. We examined the restriction phenotypes of viruses containing both hCA and sCA. Our results indicate that hCA and sCA can coassemble into the same mature core to produce infectious virus. To our knowledge, this is the first demonstration of functional coassembly of heterologous CA protein into retroviral core.

## INTRODUCTION

Coinfection of two distinct retroviruses in the same host occurs in nature. For example, a significant human population is currently coinfecting with human immunodeficiency virus type 1 (HIV-1) and HIV-2, two distinct lentiviruses (2, 19, 25). Phylogenetic analyses of the primate lentiviruses indicate that some of the viruses are recombinants of two distinct parental viruses (3, 37), indicating that the two parental viruses must coinfect the same host and infect the same target cells. Coinfection allows interactions between two viruses by several mechanisms including recombination and pseudotyping. Recombination generates a progeny different from the two parents; the best known example is that simian immunodeficiency virus  $SIV_{cpz}$ , the zoonotic precursor of HIV-1, is thought to be a recombinant of  $SIV_{rcm}$  and  $SIV_{gsn}$  (3). Pseudotyping refers to virions containing components from two different viruses. A commonly observed example of pseudotyping is the use of Env proteins from different viruses, although pseudotyping can occur with many other virion components. Pseudotyped viruses often have different properties compared with virions containing components from one virus; for example, Env-pseudotyped viruses can have a different host range (27, 40).

In all retroviruses, the major structural proteins are encoded by the *gag* gene, which is translated into the Gag polyprotein. During virus assembly, Gag coordinates the incorporation of other viral proteins and viral RNA, and interacts with the host cell machinery to facilitate the release of viral particles (15, 20, 43). In orthoretroviruses, most of the newly released particles are “immature”; virus-encoded protease cleaves the Gag polyprotein during or soon after assembly to allow the transformation from

immature to mature particles (1). This process, termed maturation, is required for the production of infectious virions. Although Gag polyproteins from different retroviruses might have only limited sequence homology, they share three similarly organized, conserved domains: matrix (MA), capsid (CA), and nucleocapsid (NC). Additionally, Gag from different retroviruses can have other domains. For example, the proteolytic cleavage of HIV-1 Gag also yields spacer peptide 1 (SP1 or p2), SP2 (p1), and p6 proteins. In the immature particles, Gag forms an approximately spherical shell underlying the membrane (51). After cleavage from the polyprotein, HIV-1 CA protein undergoes structural refolding and reassembles into a cone-shaped core that encloses the genomic RNA, NC, reverse transcriptase, integrase, and other viral and host components (1, 6, 8). It has been estimated that although each immature HIV-1 particle contains around 5000 Gag proteins, the mature shell of HIV-1 contains 1000 to 1500 CA proteins assembled into a mostly hexameric lattice (7). Therefore, only a fraction of CA proteins generated from Gag cleavage are used to form one mature virion core.

We previously demonstrated that Gag proteins from HIV-1 and HIV-2 can coassemble and complement each other's functions (5). Because only a fraction of the mature CA proteins are used to generate a core, it is possible that only the CA proteins from one of the viruses are used to form a core. Coassembly of heterologous CA proteins into a core has not previously been demonstrated; therefore, it is unclear whether such a core can be formed and whether it is capable of conducting all steps leading to a successful infection. The mature CA proteins in the virion core play important roles in the viral replication cycle (14, 44, 45, 50, 52). After entering the cells, virus uncoating occurs; CA proteins play an important role in regulating the uncoating process.

Mutations in CA can lead to acceleration or delay of the uncoating events (14); both alterations can abolish the infectivity of HIV-1. Additionally, CA can influence the ability of HIV-1 to infect nondividing cells, possibly by affecting nuclear import (12, 53). Taken together, the identity of the CA proteins can affect the biological properties of the virus, and it is possible that a coassembled core can have properties different from those of the two parental viruses.

We sought to determine whether CA proteins from two different primate lentiviruses can coassemble into a mature core to carry out all the steps necessary for infection. We envisioned three possibilities in virions with heterologous Gag proteins: first, mature heterologous CA proteins do not coassemble, and the cores consist of CA proteins from one virus (pure CA cores); second, heterologous CA proteins coassemble into a core (mixed CA core), but viruses containing mixed cores are not infectious; and third, heterologous CA proteins coassemble into a mixed core, and viruses containing mixed cores are infectious. To answer our experimental question, we exploited the inhibitory specificity of the tripartite motif 5 $\alpha$  protein from rhesus monkey (rhTRIM5 $\alpha$ ) to cores containing HIV-1 CA (hCA) but not SIV<sub>mac</sub> CA (sCA).

TRIM5 $\alpha$  is a member of the tripartite motif-containing family of proteins (35). The tripartite motif comprises a RING domain that includes two zinc finger motifs, one or two B-box domains, and a coiled-coil domain that mediates protein-protein interactions between TRIM family members (32, 41, 47). The rhTRIM5 $\alpha$  protein restricts the replication of HIV-1 and HIV-2, but not SIV<sub>mac</sub>, a virus closely related to HIV-2 (22, 41, 54, 55). Although the mechanism by which rhTRIM5 $\alpha$  restricts HIV-1 replication is not fully elucidated, recent studies suggest that rhTRIM5 $\alpha$  targets the incoming mature HIV-1 core to promote premature uncoating and possibly degradation of CA proteins (9, 42). However, the inhibition of HIV-1 infection

imposed by TRIM5 $\alpha$  can be saturated by overwhelming the system with restriction-sensitive mature viruses or virus-like particles (21, 39, 41, 54). Interestingly, TRIM5 $\alpha$  restriction cannot be saturated by adding immature virions or particles with cores consisting of restriction-insensitive CA proteins (10, 13). Furthermore, saturation of TRIM5 $\alpha$  restriction depends on the stability of the incoming HIV-1 capsid (39). These results strongly support that TRIM5 $\alpha$  recognizes CA that has undergone conformational changes or the tertiary structure of CA in the mature core.

Using N- and B-tropic murine leukemia viruses, two highly homologous virus strains that differ in their sensitivities to Fv-1 and TRIM5 $\alpha$ , it was demonstrated that coassembled viruses were sensitive to host Fv-1 restriction (26, 34, 46). The restriction imposed by rhTRIM5 $\alpha$  is specific to hCA and is relieved in HIV-1 particles that have cores containing sCA proteins (33). We hypothesize that if hCA and sCA can coassemble into the same core, TRIM5 $\alpha$  can recognize hCA present in the mixed CA core and restrict the infection of such viruses; in contrast, a population of pure CA core viruses, some with sCA cores and some with hCA cores, would generate a different restriction pattern. To determine whether hCA and sCA can coassemble to form a mixed core, we examined the TRIM5 $\alpha$  restriction phenotypes of viruses containing two different types of Gag proteins, one with hCA and the other with sCA. To ensure that most of the infection events observed were from coassembled viruses, we used two modified viruses that each harbored a debilitating *gag* mutation so that viruses derived from neither mutant could replicate efficiently. However, Gag proteins from two mutants could coassemble to allow functional complementation, thereby rescuing efficient virus replication. Our results indicate that hCA and sCA can coassemble into a mixed CA core to produce infectious virus.

## MATERIALS AND METHODS

**Plasmid construction.** Plasmids were constructed with standard molecular cloning procedures (38). The general structures of all constructs were verified by restriction mapping, and PCR-amplified regions were further characterized by DNA sequencing to avoid inadvertent mutations. For clarity, pHDV-eGFP (48) and its derivative pHIV-Thy1 (36) are referred to as hCA-gfp and hCA-thy, respectively. For clarity, HIV-1-based vectors 1-NC-thy and 1-PTAP-gfp (5), containing mutations in the NC and PTAP regions of HIV-1 Gag, respectively, are referred to as hCA-NC\*-thy and hCA-PTAP\*-gfp, respectively. By overlapping PCR and cloning, most of the CA [amino acid (aa) 1 to 204] region in hCA-NC\*-thy and hCA-PTAP\*-gfp was replaced with SIV<sub>mac</sub> CA (aa 1-202) to generate sCA-NC\*-thy and sCA-PTAP\*-gfp, respectively. Vectors sCA-thy and sCA-gfp were constructed by replacing the *AtaII-SbfI* fragments in hCA-thy and hCA-gfp respectively with the corresponding fragment in NL43-sCA; NL43-sCA contains SIV<sub>mac</sub> CA (aa 1 to 202) in place of HIV-1 CA (aa 1 to 204).

**Cells, transfections, and infections.** Cell lines 293T and 293T cells expressing TRIM5 $\alpha$  or empty plasmid have been previously described (41). These cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% calf serum, penicillin (50 U/ml), and streptomycin (50 U/ml). All cultured cells were maintained at 37°C with 5% CO<sub>2</sub>.

To generate vector-derived viruses, 293T cells were transiently transfected with an HIV-1 vector and pHCMV-G, which expresses vesicular stomatitis virus G protein (VSV-G), using the MBS Mammalian Transfection Kit (Stratagene). Viral supernatants were harvested 24 h later,

clarified through a 0.45- $\mu\text{m}$ -pore-size filter to remove cellular debris, and used immediately or stored at  $-80^{\circ}\text{C}$  prior to infection.

For infection of 293T cells expressing TRIM5 $\alpha$  or empty plasmid, 50,000 cells were plated in each well of a 24-well plate and infected 24 h later. Serial dilutions were generated from each viral stock and used to infect target cells in the presence of polybrene at a final concentration of 10  $\mu\text{g}/\text{ml}$ . Viruses were removed 2 h later, and fresh medium was added to the cells; target cells were processed 48 h postinfection, followed by flow cytometry analyses.

**Antibody staining and flow cytometry analyses.** Cells were stained with allophycocyanin-conjugated anti-Thy-1 antibody (eBioscience) and fixed with 2% paraformaldehyde prior to flow cytometry analyses. These assays were performed on a FACSCalibur apparatus (BD Biosciences), and data obtained were analyzed with Flowjo software (Tree Star). Infected cells were scored by expression of Thy or GFP marker. Multiplicity of infection (MOI) was calculated from the number of infected cells obtained from flow cytometry as previously described (11).

## RESULTS

**Strategy to examine whether CA proteins from distinct primate lentiviruses can coassemble to form infectious cores.** To determine whether CA proteins from two different viruses can coassemble into mature cores to form infectious particles, we exploited the specificity of rhTRIM5 $\alpha$  to restrict the replication of viruses containing the hCA core, but not the sCA core. We previously observed that HIV-1 and HIV-2 Gag proteins can coassemble. Therefore, we expected that HIV-1 Gag molecules containing hCA can coassemble with those

containing sCA (Fig. 1) because of the high similarity between SIV<sub>mac</sub> and HIV-2 (83% amino acid sequence identity in Gag). Coassembled viruses that contain both types of Gag proteins would have two different mature CA proteins, hCA and sCA, after proteolytic cleavage. If hCA and sCA cannot coassemble into the same core, the virus preparation would be a mixture of particles with pure CA cores. Viruses with hCA cores would be restricted by rhTRIM5 $\alpha$ , whereas those with sCA would not. Therefore, the titer of such a virus preparation would be slightly decreased by rhTRIM5 $\alpha$ . In contrast, if hCA and sCA can coassemble to form a mixed CA core, rhTRIM5 $\alpha$  would recognize and significantly restrict the infection of particles containing these cores. By determining the phenotypes of the coassembled viruses, we could determine whether hCA and sCA are coassembled in the cores of infectious particles.

**Characterization and complementation of the mutant constructs.** To ensure that most of the infectious viruses contained both Gag proteins, we introduced mutations in either the NC domain or the PTAP motif of our vectors. The NC mutation replaces the conserved CCHC/CCHC with CCHH/CCCC and drastically decreases the viral RNA encapsidation and virus titer (18). The PTAP mutation changes PTAP to LIRL and significantly reduces the virus production and virus titer (23). When coexpressed in the HIV-1 context, these two mutants can complement each other to generate infectious viruses with mostly restored infectivity (5).

To implement this strategy, we used several HIV-1-based vectors that contain *cis*-acting elements essential for viral replication and express *gag-pol*, *tat*, *rev*, and a marker gene, either *thy* or *gfp* (Fig. 2). The vector name indicates the identity of the CA, nature of the mutation, and encoded marker. For example, hCA-*thy* expresses wild-type HIV-1 *gag-pol*, sCA-*thy* expresses a functional chimeric *gag-pol* with most of the CA domain derived from SIV<sub>mac</sub>, and hCA-NC\*-

thy expresses HIV-1 *gag-pol* with a mutation in NC; additionally, these three vectors express the *thy* marker.

To measure the viral titer generated by each vector, we transfected the vector plasmid into 293T cells along with pHCMV-G, which expresses VSV-G. Viruses were harvested from transfected cells and used to infect fresh 293T cells; infected cells were later analyzed by flow cytometry. Representative flow cytometry analyses are shown in the top and middle panels of Fig. 3. As expected, the NC mutants and PTAP mutants generated drastically decreased titers compared with titers generated by hCA vectors and sCA vectors that contain functional *gag-pol*.

To ensure that Gag containing hCA and sCA can coassemble and complement each other's function, we cotransfected two constructs, one with an NC mutation and one with a PTAP mutation and examined the viral titers generated from these transfections. As shown in the bottom panels of Fig. 3, two hCA-containing or two sCA-containing Gag proteins can complement each other's function. Furthermore, hCA-containing and sCA-containing Gag proteins can also complement each other's function to generate robust viral titers. The successful complementation between vectors with heterologous CA domains provided the opportunity to study whether heterologous CA proteins coassemble into infectious viral cores.

#### **TRIM5 $\alpha$ restriction in viruses with pure CA cores consisting of either hCA or sCA.**

Several reports have demonstrated that TRIM5 $\alpha$  restriction can be saturated and the level of restriction depends on the amount of virus used for infection (4, 10, 13, 21, 39, 41, 54). To examine TRIM5 $\alpha$  restriction of virus infection, we serially diluted each viral preparation and measured viral titers generated from infecting 293T cells expressing rhTRIM5 $\alpha$  and control 293T cells expressing an empty vector; for simplicity, these cells will be referred to as TRIM5 $\alpha$  cells and control cells, respectively. We then converted the percentage of infection to MOI and

calculated the level of restriction by dividing the MOI in control cells by the MOI in TRIM5 $\alpha$  cells at a given dilution of a viral stock. Three independent experiments were performed and generated similar results; representative results of restriction in viruses derived from hCA-gfp or sCA-thy are shown in Fig. 4A and Table 1. Viruses generated from hCA-gfp alone (blue circles, Fig. 4A) were greatly restricted in TRIM5 $\alpha$  cells compared with those in control cells; consistent with the previous observations, the levels of restriction were affected by the amount of the input virus. Using serially diluted hCA-gfp viruses, we determined that the percentages of infection in TRIM5 $\alpha$  cells compared with control cells were 24% versus 95.3%, 4% versus 73.2%, and 1.3% versus 46.3%, which resulted in an 11- to 48-fold restriction. In contrast, sCA-thy-derived viruses (orange triangles, Fig. 4A) generated similar percentages of infection in TRIM5 $\alpha$  and control cells: 72.2% versus 73.8%, 52.5% versus 56.50%, 40.50% versus 40.90%, 24.8% versus 28.6%, and 11.4% versus 14% (Fig. 4A and Table 1).

We then tested the effect of infecting cells with a mixture of viruses, some containing hCA cores and the others sCA cores. Virus stocks were generated separately derived either from hCA-gfp or from sCA-thy; these two virus stocks were then added together at various ratios and used to infect TRIM5 $\alpha$  and control cells, and the infected cells in both cell lines were measured and levels of TRIM5 $\alpha$  restriction were determined. When viral stocks of hCA-gfp and sCA-thy were mixed at equal amounts, a 2- to 2.5-fold restriction was observed, whereas when viral stocks of hCA-gfp and sCA-thy were mixed at 1:2 and 1:4 ratios, very little restriction was observed in both mixtures. We reasoned that the virus population with sCA would infect both TRIM5 $\alpha$  cells and control cells equally well, whereas the virus population with hCA cores would infect TRIM5 $\alpha$  cells poorly compared with control cells. When the effects of the two populations were added together, we should observe a very mild restriction. A theoretical

simulation of this scenario is shown in Table 2. For example, if we have a mixture of viruses and half of the viruses contain hCA cores and half contain sCA cores, each infecting 5% of control cells, then 10% of the infections should be detected in control cells. If hCA virus is restricted by 50-fold in the TRIM5 $\alpha$  cells, it would yield 0.2% of infected cells. Because sCA virus is not restricted by rhTRIM5 $\alpha$ , it would yield 5% infection; together, a total of 5.2% of the infections should be detected. The level of restriction is calculated by comparing the control cell infection (10%) with the TRIM5 $\alpha$  cell infection (5.2%), which yields approximately 2-fold restriction. The level of restriction would decrease when the relative amounts of sCA virus increase (Table 2). These predictions are consistent with our results of virus populations containing pure CA cores.

**TRIM5 $\alpha$  restriction phenotypes of viruses containing both HIV-1 and SIV<sub>mac</sub> CA proteins.** To determine whether heterologous CA proteins can coassemble into cores of infectious viruses, we examined the restriction phenotypes of viruses generated by cotransfected vectors hCA-NC\*-thy and sCA-PTAP\*-gfp. A total of three independent experiments were performed that yielded similar results; data from one set of representative experiments are shown in Table 3 and Fig 4B. For controls, we also examined the restriction phenotypes of viruses generated from cotransfecting at a 1:1 ratio either two hCA-containing vectors (hCA-NC\*-thy and sCA-PTAP\*-gfp), or two sCA-containing vectors (sCA-NC\*-thy and sCA-PTAP\*-gfp). As expected, we observed strong rhTRIM5 $\alpha$  restriction when both vectors used in the complementation system contained hCA (blue circles), and little, if any, restriction when both vectors contained sCA (orange triangles) (Fig. 4B).

The phenotypes of viruses containing both hCA and sCA proteins are also shown in Fig. 4B. We observed significant restriction in viruses generated by cotransfecting hCA-NC\*-thy

and sCA-PTAP\*-gfp. However, the levels of restriction are lower than those in viruses containing only hCA proteins (blue circles, Fig. 4B). When hCA- and sCA-expressing vectors were mixed at equal amounts, the resulting viruses were restricted 3.6- to 17.3-fold by rhTRIM5 $\alpha$  cells, depending on the titer of infection (pink squares, Fig. 4B). Even when hCA-expressing vectors were present at 20% of the mixture, the resulting viruses were restricted 4.4- to 10.5-fold by rhTRIM5 $\alpha$  cells depending on the titer of infection (black squares, Fig. 4B). These results are in sharp contrast to those shown in Fig. 4A, when pure CA core viruses from separate transfection were mixed together prior to infection. First, these coassembled Gag viruses had far greater levels of restriction compared with the pure CA core viruses in Fig 4A, which had a maximum of only 2- to 2.5-fold restriction in the 1:1 (hCA: sCA) mixture. Additionally, the levels of restriction of coassembled Gag viruses are heavily influenced by the amounts of input viruses, similar to the restriction kinetics of HIV-1, and are very different from those of pure CA core viruses (Fig. 4A). These results support the conclusion that hCA and sCA can coassemble into the same mature cores and such cores are susceptible to rhTRIM5 $\alpha$  restriction. Furthermore, cores containing coassembled hCA and sCA are capable of completing steps of HIV-1 replication to lead to successful infection.

## DISCUSSION

In this study, we exploited the specificity of rhesus TRIM5 $\alpha$  for hCA in the mature viral core to demonstrate that CA proteins from HIV-1 and SIV<sub>mac</sub> can coassemble into the same core to generate infectious viruses. To our knowledge, this is the first demonstration that CA proteins from distinct retroviruses can coassemble into an infectious mature core.

Primate lentiviruses consist of HIV-1, HIV-2, and various SIV strains isolated from more than 30 nonhuman primate species; most of these viruses can be assigned to one of the six approximately equidistant phylogenetic lineages (37). Generally, the Gag proteins from viruses of different phylogenetic lineages are around 50% identical in amino acid sequences, whereas the Gag proteins from viruses of the same phylogenetic lineage are usually more than 80% identical in amino acid sequence (HIV databases, <http://www.hiv.lanl.gov>). HIV-2 and SIV<sub>mac</sub> belong to the same phylogenetic lineage, whereas HIV-1 belongs to a different lineage. In terms of amino acid sequence, the Gag proteins of HIV-2 and SIV<sub>mac</sub> share 83% identity, whereas these two proteins share 54% and 53% identity with HIV-1 Gag, respectively. Among the Gag domains of primate lentiviruses, CA usually has the highest similarity. The CA protein of HIV-2 and SIV<sub>mac</sub> share 90% amino acid identity, whereas these two proteins share 68% and 66% identity with HIV-1 CA protein. The overall higher conservation of the CA domain relative to other domains is probably due to the evolutionary constraints imposed by the multiple roles CA plays during retrovirus replication, both as a Gag domain and as a mature protein.

Our current understanding of virus maturation is that core formation is not simply condensation of the CA domain of the Gag lattice after proteolytic cleavage. Rather, a second assembly step, including the refolding of the mature CA protein and formation of a hexamer CA lattice, is involved in the formation of the cone-shaped core (49). The structure and protein-protein interactions of mature retrovirus cores are just beginning to be elucidated. The core of HIV-1 is proposed to be a fullerene structure composed of 200-300 CA hexamers and 12 CA pentamers (17, 30). Recent structural analyses revealed that there are three important protein-protein interaction interfaces in the mature core (16, 28, 29). Helices 1 to 3 in the N-terminal domain of CA mediate the N-terminal-N-terminal hexamerization interaction interface. Helix 9

in the C-terminal domain primarily mediates the C-terminal-C-terminal dimerization interaction. Helix 4 in the N-terminal domain and helices 8 to 11 in the C-terminal domain are involved in the N-terminal-C-terminal interaction. Therefore, extensive interactions between neighboring CA molecules in the hexameric lattice are required in forming a mature core. Although our current knowledge does not extend to the detailed CA-CA sidechain interactions in immature or mature particles, mutagenesis analyses have identified a number of amino acids potentially involved in these interactions. Mutations R18A/N21A, A22D, E28A/E29A, M39D, A42D, D51A, T54A/N57A, and K70A have been shown to reduce or alter mature core formation, whereas mutations E75A/E76A, R100A/S102A, T107A/T108A, T110A/Q112A, K158ADQ, W184A, M18A, and D197AEN diminish immature particle production (50). Most of these amino acids are conserved between hCA and sCA. However, M39, T54, K70, R100, T110, and Q112 in hCA are changed to G, Q, R, S, S and D in sCA, respectively. These differences appear to be tolerated and do not prevent the coassembly of Gag proteins containing CA domains from HIV-1 and SIV<sub>mac</sub> or the formation of mature cores from these heterologous CA proteins. What remains unknown is the arrangement of these heterologous CA proteins in the mature core. For example, it is not clear whether these two different CA proteins are evenly distributed in the core and whether these two different CA proteins form mixed hexamers.

We demonstrated that viruses containing hCA and sCA were restricted by rhTRIM5 $\alpha$  in a virus-titer-dependent manner. However, the level of rhTRIM5 $\alpha$  restriction in these viruses was less potent than in viruses containing pure hCA cores at a comparable infection rate (Fig. 4B). Furthermore, the levels of restriction are directly correlated with the amount of hCA-containing vector used in transfection. For example, when control cells were infected at about the 55% level, the restriction in hCA cores is about 27-fold (Fig. 4B and Table 3); when the hCA and sCA

ratios are 1:1, and 1:2, the restriction is about 11-fold and 8-fold, respectively; and when the hCA and sCA ratio is 1:4, the restriction is estimated to be 6-fold. Therefore, these mixed-core viruses have an intermediate phenotype and the restriction levels are in proportion to the amounts of hCA in the mixture. These results reveal that the number of TRIM5 $\alpha$  binding sites in the cores is an important limiting factor of restriction. It is possible that multiple TRIM5 $\alpha$  binding is required to achieve restriction or that TRIM5 $\alpha$  finding the target hCA is a rate-limiting step. Therefore, decreasing the number of target hCA proteins causes the reduction of TRIM5 $\alpha$  restriction efficiency. Experimental evidence indicates that TRIM5 $\alpha$  is a trimer, and it has been proposed that the three molecules of the trimer can fit into three binding pockets in a CA hexamer or between hexamers (24, 31). If each of the molecules in the trimer needs to bind a pocket to be functional, and hCA and sCA assemble randomly, then the probability of a TRIM5 $\alpha$  trimer finding all three binding pockets to be suitable should reduce nonlinearly with the decrease in the amounts of hCA. For example, when hCA and sCA are mixed in 1:1, 1:2, and 1:4 ratios, the probability of a trimer finding three suitable binding sites should be 1 in 8 ( $2^3$ ), 1 in 27 ( $3^3$ ), and 1 in 125 ( $5^3$ ), respectively. These probabilities are reduced even more drastically if more than one hCA protein is required to form each binding pocket. In either scenario, binding sites to TRIM5 $\alpha$  trimers in the core are not linear to the amounts of hCA proteins in the core. Our data indicate a near-linear relationship between the proportion of hCA in the virus and the restriction level, thereby suggesting either that all three TRIM5 $\alpha$  molecules in the trimer are not required to bind to the core to be functional, or that hCA and sCA are not distributed randomly in the core because homologous CA proteins have stronger affinity to each other than to heterologous CA proteins. To distinguish between these two possibilities requires further

advances in our understanding of TRIM5 $\alpha$ -CA binding and the assembly of heterologous CA proteins in virus cores.

In this report, we illustrate that sufficient features are conserved between CA proteins from HIV-1 and SIV<sub>mac</sub> to allow coassembly of infectious virus cores. The mixed cores have an intermediate phenotype in terms of restriction by TRIM5 $\alpha$ . One possible implication is that virus could use this mechanism to lower the efficiency of the host innate defense system to initiate infection. These experiments are the first to demonstrate that heterologous CA proteins can coassemble into mature cores of infectious viruses and have implications to virus evolution. These studies also provide insights into the mechanisms of TRIM5 $\alpha$  restriction and virus assembly.

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#### REFERENCES

1. Adamson, C. S., and E. O. Freed. 2007. Human immunodeficiency virus type 1 assembly, release, and maturation. *Advances in Pharmacology* 55:347-387.
2. Arien, K. K., A. Abraha, M. E. Quinones-Mateu, L. Kestens, G. Vanham, and E. J. Arts. 2005. The replicative fitness of primary human immunodeficiency virus type 1 (HIV-1) group M, HIV-1 group O, and HIV-2 isolates. *J. Virol.* 79:8979-8990.
3. Bailes, E., F. Gao, F. Bibollet-Ruche, V. Courgnaud, M. Peeters, P. A. Marx, B. H. Hahn, and P. M. Sharp. 2003. Hybrid origin of SIV in chimpanzees. *Science* 300:1713.
4. Besnier, C., Y. Takeuchi, and G. Towers. 2002. Restriction of lentivirus in monkeys. *Proc. Natl. Acad. Sci. USA* 99:11920-11925.
5. Boyko, V., M. Leavitt, R. Gorelick, W. Fu, O. Nikolaitchik, V. K. Pathak, K. Nagashima, and W.-S. Hu. 2006. Coassembly and complementation of Gag proteins from HIV-1 and HIV-2, two distinct human pathogens. *Molecular Cell* 23:281.
6. Briggs, J. A. G., K. Grunewald, B. Glass, F. Forster, H.-G. Krausslich, and S. D. Fuller. 2006. The mechanism of HIV-1 core assembly: insights from three dimensional reconstructions of authentic virions. *Structure* 14:15-20.
7. Briggs, J. A. G., M. N. Simon, I. Gross, H.-G. Krausslich, S. D. Fuller, V. M. Vogt, and M. C. Johnson. 2004. The stoichiometry of Gag protein in HIV-1. *Nature Structure & Molecular Biology* 11:672-675.
8. Briggs, J. A. G., T. Wilk, R. Welker, H.-G. Krausslich, and S. D. Fuller. 2003. Structural organization of authentic, mature HIV-1 virions and cores. *EMBO J.* 22:1707-1716.
9. Chatterji, U., M. D. Bobardt, P. Gaskill, D. Sheeter, H. Fox, and P. A. Gally. 2006. Trim5alpha accelerates degradation of cytosolic capsid associated with productive HIV-1 entry. *J Biol Chem* 281:37025-33.

10. Cowan, S., T. Hatzioannou, T. Cunningham, M. A. Muesing, H. G. Gottlinger, and P. D. Bieniasz. 2002. Cellular inhibitors with Fv1-like activity restrict human and simian immunodeficiency virus tropism. *Proc. Natl. Acad. Sci. USA* 99:11914-11919.
11. Dang, Q., J. Chen, D. Unutmaz, J. M. Coffin, V. K. Pathak, D. Powell, V. N. KewalRamani, F. Maldarelli, and W. S. Hu. 2004. Nonrandom HIV-1 infection and double infection via direct and cell-mediated pathways. *Proc. Natl. Acad. Sci. USA* 101:632-637.
12. Dismuke, D. J., and C. Aiken. 2006. Evidence for a functional link between uncoating of the human immunodeficiency virus type 1 core and nuclear import of the Viral preintegration complex. *J. Virol.* 80:3712-3720.
13. Dodding, M. P., M. Bock, M. W. Yap, and J. P. Stoye. 2005. Capsid processing requirements for abrogation of Fv1 and Ref1 restriction. *J. Virol.* 79:10571-10577.
14. Forshey, B. M., U. von Schwedler, W. I. Sundquist, and C. Aiken. 2002. Formation of a human immunodeficiency virus type 1 core of optimal stability is crucial for viral replication. *J. Virol.* 76:5667-5677.
15. Freed, E. O. 2002. Viral late domains. *J. Virol.* 76:4679-4687.
16. Ganser-Pornillos, B. K., A. Cheng, and M. Yeager. 2007. Structure of full-length HIV-1 CA: a model for the mature capsid lattice. *Cell* 131:70-79.
17. Ganser-Pornillos, B. K., S. Li, V. Klishko, J. T. Finch, and W. I. Sundquist. 1999. Assembly and analysis of conical models for the HIV-1 core. *Science* 283:80-83.
18. Gorelick, R. J., T. D. Gagliardi, W. J. Bosche, T. A. Wiltrout, L. V. Coren, D. J. Chabot, J. D. Lifson, L. E. Henderson, and L. O. Arthur. 1999. Strict conservation of the retroviral nucleocapsid protein zinc finger is strongly influenced by its role in viral

- infection processes: characterization of HIV-1 particles containing mutant nucleocapsid zinc-coordinating sequences. *Virology* 256:92.
19. Gottlieb, G. S., P. S. Sow, S. E. Hawes, I. Ndoye, A. M. Coll-Seck, M. E. Curlin, C. W. Critchlow, N. B. Kiviat, and J. I. Mullins. 2003. Molecular epidemiology of dual HIV-1/HIV-2 seropositive adults from Senegal, West Africa. *AIDS Res. Hum. Retroviruses* 19:575-584.
  20. Gottlinger, H., G. 2001. The HIV-1 assembly machine. *AIDS* 15:S13-S20.
  21. Hatziioannou, T., S. Cowan, S. P. Goff, P. D. Bieniasz, and G. J. Towers. 2003. Restriction of multiple divergent retroviruses by Lv1 and Ref1. *Embo J* 22:385-94.
  22. Hatziioannou, T., D. Perez-Caballero, A. Yang, S. Cowan, and P. D. Bieniasz. 2004. Retrovirus resistance factors Ref1 and Lv1 are species-specific variants of TRIM5alpha. *Proc Natl Acad Sci U S A* 101:10774-9.
  23. Huang, M., J. M. Orenstein, M. A. Martin, and E. O. Freed. 1995. p6Gag is required for particle production from full-length human immunodeficiency virus type 1 molecular clones expressing protease, p. 6810-6818, vol. 69.
  24. Javanbakht, H., W. Yuan, D. F. Yeung, B. Song, F. Diaz-Griffero, Y. Li, X. Li, M. Stremlau, and J. Sodroski. 2006. Characterization of TRIM5[alpha] trimerization and its contribution to human immunodeficiency virus capsid binding. *Virology* 353:234-246.
  25. Kannangai, R., R. V. Shaji, S. Ramalingam, M. V. Jesudason, O. C. Abraham, R. George, A. P. Shanmugam, D. H. Schwartz, and G. Sridharan. 2003. HIV-2 subtype circulating in India (south). *J. Acquir. Immune Defic. Syndr.* 33:219-222.

26. Kashmiri, S. V., A. Rein, R. H. Bassin, B. I. Gerwin, and S. Gisselbrecht. 1977. Donation of N- or B-tropic phenotype to NB-tropic murine leukemia virus during mixed infections. *J Virol* 22:626-33.
27. Landau, N. R., K. A. Page, and D. R. Littman. 1991. Pseudotyping with human T-cell leukemia virus type I broadens the human immunodeficiency virus host range. *J. Virol.* 65:162-169.
28. Lanman, J., T. T. Lam, S. Barnes, M. Sakalian, M. R. Emmett, A. G. Marshall, and P. E. Prevelige. 2003. Identification of novel interactions in HIV-1 capsid protein assembly by high-resolution mass spectrometry. *Journal of Molecular Biology* 325:759-772.
29. Lanman, J., T. T. Lam, M. R. Emmett, A. G. Marshall, M. Sakalian, and P. E. P. Jr. 2004. Key interactions in HIV-1 maturation identified by hydrogen-deuterium exchange. *Nature Structure & Molecular Biology* 11:676-677.
30. Li, S., C. P. Hill, W. I. Sundquist, and J. T. Finch. 2000. Image reconstructions of helical assemblies of the HIV-1 CA protein. *Nature* 407:409-413.
31. Mische, C. C., H. Javanbakht, B. Song, F. Diaz-Griffero, M. Stremlau, B. Strack, Z. Si, and J. Sodroski. 2005. Retroviral restriction factor TRIM5{alpha} is a trimer. *J. Virol.* 79:14446-14450.
32. Nisole, S., J. P. Stoye, and A. Saib. 2005. TRIM family proteins: retroviral restriction and antiviral defence. *Nat Rev Microbiol* 3:799-808.
33. Owens, C. M., P. C. Yang, H. Gottlinger, and J. Sodroski. 2003. Human and simian immunodeficiency virus capsid proteins are major viral determinants of early, postentry replication blocks in simian cells. *J. Virol.* 77:726-731.

34. Rein, A., S. V. Kashmiri, R. H. Bassin, B. L. Gerwin, and G. Duran-Troise. 1976. Phenotypic mixing between N- and B-tropic murine leukemia viruses: infectious particles with dual sensitivity to Fv-1 restriction. *Cell* 7:373-9.
35. Reymond, A., G. Meroni, A. Fantozzi, G. Meria, S. Cairo, L. Luzi, D. Riganelli, E. Zanaria, S. Messali, S. Cainarca, A. Guffanti, S. Minucci, P. G. Pelicci, and A. Ballobio. 2001. The tripartite motif family identifies cell compartments. *EMBO J.* 20:2140-2151.
36. Rhodes, T., O. Nikolaitchik, J. Chen, D. Powell, and W. S. Hu. 2005. Genetic recombination of human immunodeficiency virus type I in one round of viral replication: effects of genetic distance, target cells, accessory genes, and lack of high negative interference in crossover events. *J. Virol.* 79:1666-1677.
37. Salemi, M., T. De Oliveira, V. Courgnaud, V. Moulton, B. Holland, S. Cassol, W. M. Switzer, and A.-M. Vandamme. 2003. Mosaic genomes of the six major primate lentivirus lineages revealed by phylogenetic analyses. *J. Virol.* 77:7202-7213.
38. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
39. Shi, J., and C. Aiken. 2006. Saturation of TRIM5[alpha]-mediated restriction of HIV-1 infection depends on the stability of the incoming viral capsid. *J. Virol.* 350:493-500.
40. Spector, D. H., E. Wade, D. A. Wright, V. Koval, C. Clark, D. Jaquish, and S. A. Spector. 1990. Human immunodeficiency virus pseudotypes with expanded cellular and species tropism. *J. Virol.* 64:2298-2308.
41. Stremlau, M., C. M. Owens, M. J. Perron, M. Kiessling, P. Autissier, and J. Sodroski. 2004. The cytoplasmic body component TRIM5 $\alpha$  restricts HIV-1 infection in Old World Monkeys. *Nature* 427:848-853.

42. Stremlau, M., M. Perron, M. Lee, Y. Li, B. Song, H. Javanbakht, F. Diaz-Griffero, D. J. Anderson, W. I. Sundquist, and J. Sodroski. 2006. Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5 $\alpha$  restriction factor. *PNAS* 103:5514-5519.
43. Swanstrom, R., and J. W. S. Wills, 1997 #186}. 1997. Synthesis, assembly, and processing of viral proteins. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
44. Tang, S., T. Murakami, B. E. Agresta, S. Campbell, E. O. Freed, and J. G. Levin. 2001. Human immunodeficiency virus type 1 N-terminal capsid mutants that exhibit aberrant core morphology and are blocked in initiation of reverse transcription in infected cells. *J. Virol.* 75:9357-9366.
45. Tang, S., T. Murakami, N. Cheng, A. C. Steven, E. O. Freed, and J. G. Levin. 2003. Human immunodeficiency virus type 1 N-terminal capsid mutants containing cores with abnormally high levels of capsid protein and virtually no reverse transcriptase. *J. Virol.* 77:12592-12602.
46. Towers, G., M. Bock, S. Martin, Y. Takeuchi, J. P. Stoye, and O. Danos. 2000. A conserved mechanism of retrovirus restriction in mammals. *Proc Natl Acad Sci U S A* 97:12295-9.
47. Towers, G. J. 2007. The control of viral infection by tripartite motif proteins and cyclophilin A. *Retrovirology* 4:40-49.
48. Unutmaz, D., V. N. KewalRamani, S. Marmon, and D. R. Littman. 1999. Cytokine signals are sufficient for HIV-1 infection of resting human T lymphocytes. *J. Exp. Med.* 189:1735-46.

49. von Schwedler, U., T. Stemmler, V. Klishko, S. Li, K. Albertine, D. Davis, and W. I. Sundquist. 1998. Proteolytic refolding of the HIV-1 capsid protein amino-terminus facilitates viral core assembly. *EMBO J.* 17:1555-1568.
50. von Schwedler, U. K., K. M. Stray, J. E. Garrus, and W. I. Sundquist. 2003. Functional surfaces of the human immunodeficiency virus type 1 capsid protein. *J. Virol.* 77:5439-5450.
51. Wilk, T., I. Gross, B. E. Gowen, T. Rutten, F. de Haas, R. Welker, H.-G. Krausslich, P. Boulanger, and S. D. Fuller. 2001. Organization of immature human immunodeficiency virus type 1. *J. Virol.* 75:759-771.
52. Yamashita, M., and M. Emerman. 2004. Capsid is a dominant determinant of retrovirus infectivity in nondividing cells. *J Virol* 78:5670-8.
53. Yamashita, M., O. Perez, T. J. Hope, and M. Emerman. 2007. Evidence for direct involvement of the capsid protein in HIV infection of nondividing cells. *PLoS Pathog* 3:1502-10.
54. Yap, M. W., S. Nisole, C. Lynch, and J. P. Stoye. 2004. Trim5alpha protein restricts both HIV-1 and murine leukemia virus. *Proc Natl Acad Sci U S A* 101:10786-91.
55. Ylinen, L. M. J., Z. Keckesova, S. J. Wilson, S. Ranasinghe, and G. J. Towers. 2005. Differential restriction of human immunodeficiency virus type 2 and simian immunodeficiency virus SIVmac by TRIM5 $\alpha$  alleles. *J. Virol.* 79:11580-11587.

## FIGURE LEGENDS

FIG. 1. Strategy to examine whether CA proteins from HIV-1 (hCA) and SIV<sub>mac</sub> (sCA) can coassemble into infectious mature cores. HIV-1 vectors encoding hCA or sCA and containing either a debilitating NC or PTAP mutation were cotransfected into 293T cells. Coassembly and functional complementation of the two mutant Gag proteins result in the production of infectious virus particles. After proteolytic cleavage during virus maturation, the coassembled viruses contain both mature hCA and sCA. It is possible that hCA and sCA cannot coassemble into the same core to generate infectious viruses, in which case the infectious virus population is a mixture of particles with pure cores consisting of either hCA or sCA. It is also possible that hCA and sCA can coassemble into the same core and viruses containing these cores are infectious. To distinguish between these two possibilities, the virus preparation was used to infect 293T cells expressing rhTRIM5 $\alpha$  or control 293T cells expressing the empty plasmid, and the levels of infection were compared to determine the effect of rhTRIM5 $\alpha$  restriction on virus infectivities.

FIG. 2. General structure of HIV-1-based vectors. These vectors express *gag-pol*, *tat*, *rev*, and a marker gene, either *gfp* or *thy*. The Gag domains MA, CA, NC, and p6 are indicated. sCA represents CA domains from SIV<sub>mac</sub>. The asterisk indicates the location of the mutation.

FIG. 3. Representative flow cytometry analyses of infection by various viruses. X-axis, Thy expression; y-axis, GFP expression. Top panel, infection by viruses generated by wild-type or mutant vectors containing hCA. Middle panel, infection by viruses generated by wild-type or

mutant vectors containing sCA. Bottom panel, infection by viruses generated from cotransfection of vectors containing an NC or PTAP mutation.

FIG. 4. Restriction patterns of viruses. (A). Representative results of TRIM5 $\alpha$  restriction on viruses containing pure cores. Separately produced viruses were mixed together at various ratios and the restriction levels of such virus mixtures were determined. Three sets of independent experiments were performed and yielded similar results, in all experiments, the mixed viruses never achieved more than 3-fold of restriction. (B) Representative results of TRIM5 $\alpha$  restriction in viruses produced by cotransfection of NC or PTAP mutants with homologous or heterologous CA proteins. All three independent experiments generated similar results. In each experiment, viruses containing only hCA had the highest restriction, whereas viruses containing only sCA did not have significant restriction. Among the mixed viruses, fold of restriction decreased with lower amounts of hCA. In the three sets of experiments, when the control cells were infected at 50%, and hCA- and sCA-containing plasmids were transfected at 1:1, 1:2, and 1:4 ratios, we estimated that TRIM5 $\alpha$  restricted the virus titers 11-13, 7-9, and 5-8 fold, respectively. X-axis, percentage of infection of the virus dilution in control 293T cells; y-axis, fold restriction calculated by dividing the MOI in control cells with the MOI in rhTRIM5 $\alpha$  cells.

TABLE 1. TRIM5 $\alpha$  restriction of mixture of separately produced viruses

Mixture of virus stocks	Dilution	TRIM5 $\alpha$ cells		Control cells		Fold restriction
		Infection %*	MOI	Infection %*	MOI	
hCA-gfp	1	24.0	0.27	95.3	3.05	11.1
	2	4.0	0.04	73.2	1.32	32.3
	4	1.3	0.01	46.3	0.62	47.5
sCA-Thy	1	73.8	1.34	72.2	1.28	1.0
	2	56.5	0.83	52.5	0.74	0.9
	4	40.9	0.53	40.5	0.52	1.0
	8	28.6	0.34	24.8	0.28	0.9
	16	14.0	0.15	11.4	0.12	0.8
hCA-gfp virus plus	1	56.2	0.83	82.8	1.76	2.1
sCA-thy virus at	2	36.1	0.45	66.9	1.11	2.5
1:1 ratio	4	23.0	0.26	47.9	0.65	2.5
	8	13.3	0.14	28.6	0.34	2.4
	16	6.3	0.07	14.7	0.16	2.4
	1	66.6	1.10	73.3	1.32	1.2
hCA-gfp virus plus	2	42.0	0.54	48.3	0.66	1.2
sCA-thy virus at	4	23.3	0.27	33.5	0.41	1.5
1:2 ratio	8	13.4	0.14	18.9	0.21	1.5
	16	6.2	0.06	8.2	0.09	1.3
	1	53.7	0.77	61.3	0.95	1.2
hCA-gfp virus plus	2	41.7	0.54	44.2	0.58	1.1
sCA-thy virus at	4	25.9	0.30	30.1	0.36	1.2
1:4 ratio	8	13.9	0.15	16.3	0.18	1.2
	16	6.3	0.07	7.3	0.08	1.2

\* Infected cells were scored by the expression of either Thy or GFP markers.

TABLE 2. Simulation of restriction in mixed viral populations containing pure cores

Mixture of hCA virus : sCA virus	Percentage of infection in control cells			Percentage of infection in TRIM5 $\alpha$ -expressing cells <sup>a</sup>			Fold restriction <sup>b</sup>
	From hCA	From sCA	Total	From hCA	From sCA	Total	
1:1	5%	5%	10%	0.125%	5%	5.125%	~ 2
1:2	5%	10%	15%	0.125%	10%	10.125%	~ 1.5
1:4	5%	20%	25%	0.125%	20%	20.125%	~ 1.125

<sup>a</sup>An assumption of 50-fold restriction is made.

<sup>b</sup>For simplicity, percentages of infected cells instead of MOIs are used to calculate restriction in these examples.

TABLE 3. TRIM5 $\alpha$  restriction of viruses generated from cotransfection of two vectors

Cotransfected viral vectors	Dilutions	TRIM5 $\alpha$ cells		Control cells		Fold restriction
		Infection %*	MOI	Infection %*	MOI	
hCA-NC*-thy + hCA-PTAP*-gfp	1	27.2	0.32	92.1	2.54	8.0
1:1	2	8.1	0.08	77.8	1.50	17.8
	4	2.9	0.03	54.9	0.80	27.1
sCA-NC*-thy + sCA-PTAP*-gfp	1	77.6	1.50	76.1	1.43	1.0
1:1	2	56.3	0.83	53.4	0.76	0.9
	4	36.1	0.45	33.8	0.41	0.9
	8	21.8	0.25	16.0	0.17	0.7
hCA-NC*-thy + sCA-PTAP*-gfp	1	48.8	0.67	90.7	2.38	3.6
1:1	2	16.6	0.18	78.4	1.53	8.4
	4	7.7	0.08	58.5	0.88	11.0
	8	2.2	0.02	31.9	0.38	17.3
hCA-NC*-thy + sCA-PTAP*-gfp	1	42.1	0.55	89.5	2.25	4.1
1:2	2	23.3	0.27	76.1	1.43	5.4
	4	9.7	0.10	55.8	0.82	8.0
	8	3.5	0.04	35.8	0.44	12.4
hCA-NC*-thy + sCA-PTAP*-gfp	1	21.7	0.24	66.2	1.08	4.4
1:4	2	6.4	0.07	44.2	0.58	8.8
	4	2.7	0.03	24.9	0.29	10.5

\* Infected cells were scored by the expression of either Thy or GFP markers.

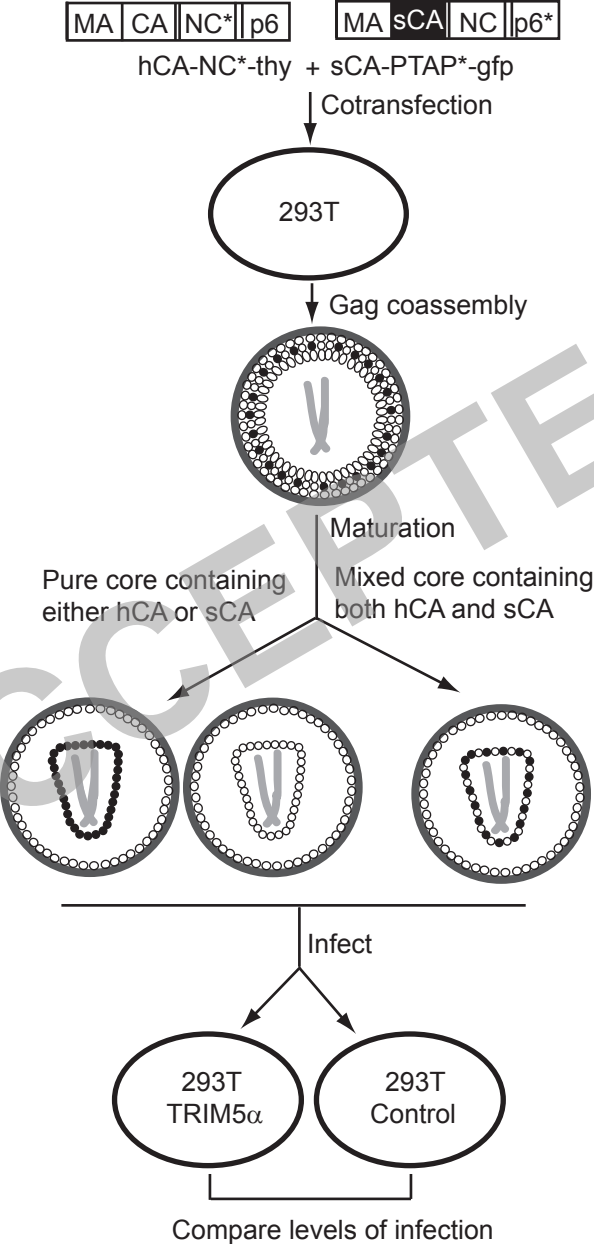


Fig. 1

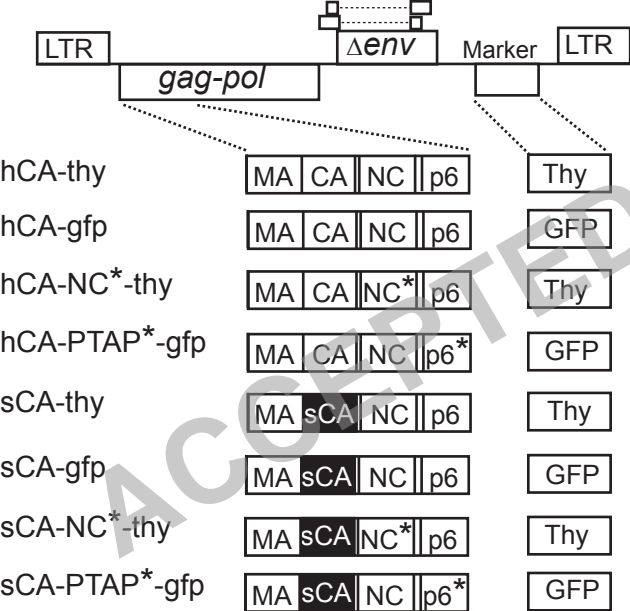


Fig. 2

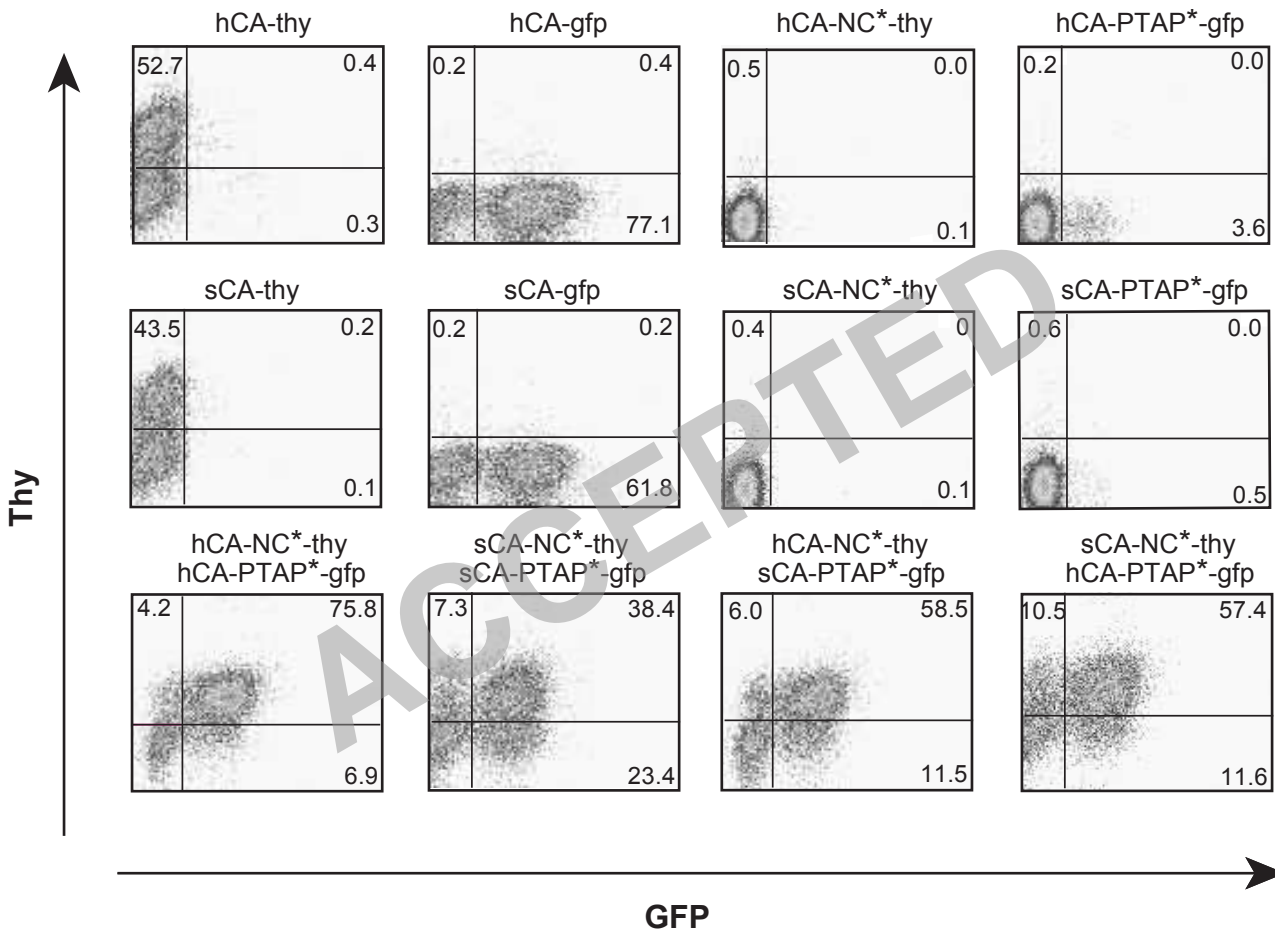
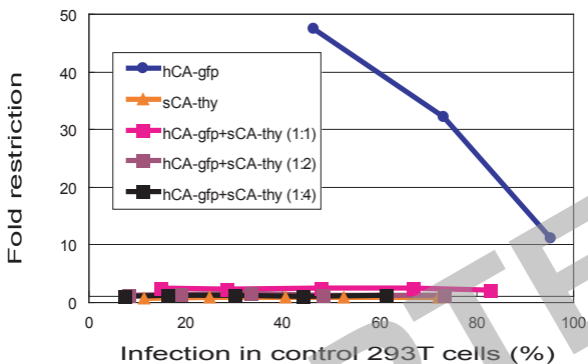


Fig. 3

### A. Viruses containing pure cores



### B. Viruses produced by cotransfection

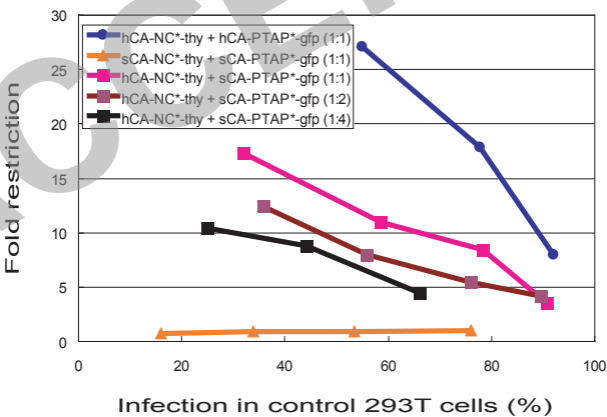


Fig. 4