Published in final edited form as:

Nat Struct Mol Biol. 2009 October ; 16(10): 1036–1042. doi:10.1038/nsmb.1667.

Active site remodeling switches HIV specificity of antiretroviral TRIMCyp

Amanda J Price^{1,4}, Flavia Marzetta^{2,4}, Michael Lammers¹, Laura M J Ylinen², Torsten Schaller², Sam J Wilson^{2,3}, Greg J Towers², and Leo C James¹

¹Protein and Nucleic Acid Chemistry Division, Medical Research Council Laboratory of Molecular Biology, Cambridge, UK.

²Division of Infection and Immunity, Medical Research Council Centre for Medical Molecular Virology, University College London, London, UK.

Abstract

TRIMCyps are primate antiretroviral proteins that potently inhibit HIV replication. Here we describe how rhesus macaque TRIMCyp (RhTC) has evolved to target and restrict HIV-2. We show that the ancestral cyclophilin A (CypA) domain of RhTC targets HIV-2 capsid with weak affinity, which is strongly increased in RhTC by two mutations (D66N and R69H) at the expense of HIV-1 binding. These mutations disrupt a constraining intramolecular interaction in CypA, triggering the complete restructuring (>16 Å) of an active site loop. This new configuration discriminates between divergent HIV-1 and HIV-2 loop conformations mediated by capsid residue 88. Viral sensitivity to RhTC restriction can be conferred or abolished by mutating position 88. Furthermore, position 88 determines the susceptibility of naturally occurring HIV-1 sequences to restriction. Our results reveal the complex molecular, structural and thermodynamic changes that underlie the ongoing evolutionary race between virus and host.

Tripartite motif–encoding proteins, or TRIMs, are a family of around 70 proteins in the human genome with an emerging role in innate immunity¹. They encode a tripartite motif comprising a RING domain, one or two B-boxes and a coiled coil that mediates dimerization². One of the most widely studied TRIMs is the antiretroviral factor TRIM5 α , which in Old World monkeys restricts the replication of HIV-1 but whose human ortholog is ineffective against this virus³. The mechanism by which primate TRIM5 α targets and restricts HIV-1 replication is of considerable interest. Previous experiments have shown that the N-terminal tripartite motif domains provide the effector functions of TRIM5 α , whereas the C-terminal PRYSPRY domain is required for binding the viral capsid^{4–7}.

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Correspondence should be addressed to G.J.T. (g.towers@ucl.ac.uk) or L.C.J. (lcj@mrc-lmb.cam.ac.uk).

³Present addresses: Viral Pathogens and Biosafety Unit, Division of Immunology, Transplantation and Infectious Diseases, San Raffaele Scientific Institute, Milano, Italy (F.M.); Aaron Diamond AIDS Research Center, Howard Hughes Medical Institute and the Rockefeller University, New York, New York, USA (S.J.W.).

⁴These authors contributed equally to this work.

AUTHOR CONTRIBUTIONS A.J.P. and F.M. performed experiments and analyzed data; M.L., L.M.J.Y., T.S. & S.J.W. performed additional experiments; G.J.T. and L.C.J. analyzed data and wrote the manuscript.

Accession codes. Protein Data Bank: Coordinates for HIV-2 and RhTC have been deposited with accession codes 2WLX and 2WLW, respectively.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

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TRIMCyp is a variant of TRIM5 α in which CypA replaces the PRYSPRY domain^{8,9}. CypA is a *cis-trans* peptidyl-prolyl isomerase that is known to bind the N-terminal domain of HIV-1 capsid and is required for efficient viral replication in human cells¹⁰⁻¹³. The CypA cDNA has been inserted into the TRIM5 locus by retrotransposition of a CypA-encoding mRNA. Initially, the TRIMCyp fusion was thought to be unique to the New World owl monkey; however, a second independently evolved TRIMCyp was recently discovered in Old World macaques¹⁴⁻¹⁶. Independent genesis is indicated by different locations of the CypA cDNA in the TRIM5 loci of Old and New World monkeys. It is assumed that selective pressure from pathogenic lentiviral infections has led to the fixation of these modified TRIM5 genes in primates. The use of CypA as a virus-binding domain for TRIMCyps is unexpected, as CypA is thought to bind a restricted set of lentiviruses. For instance, human CypA is not thought to bind HIV-2, nor is it seemingly required for its replication¹⁷. However, rhesus TRIMCyp (RhTC) potently restricts HIV-2 but not HIV-1 (refs. ^{15,16}).

Here we have used biophysical analysis, X-ray crystallography, structure-guided mutagenesis and viral infectivity assays to show how RhTC has evolved to target HIV-2, and we define key residues in both CypA and the viral capsid that control binding susceptibility and sensitivity to restriction. Our data reveal molecular details of host virus counterevolution and demonstrate how apparently small changes in protein sequence lead to significant structural changes with important consequences for virus replication.

RESULTS

CypA has previously undetected affinity for HIV-2

RhTC has evolved from exons encoding the tripartite domains of TRIM5 α and a retrotransposed CypA cDNA. Such retrotransposed cDNAs can serve as the starting point for the evolution of new protein function but only if they provide a selective advantage. We reasoned that CypA may therefore have an existing low affinity for the HIV-2 capsid that could serve as the starting point for an HIV-2-targeted restriction factor. Previous characterization of CypA using isothermal titration calorimetry (ITC) has demonstrated binding only to HIV-1 (ref. ¹⁸); however, recent technical advances (ITC200) have increased instrument sensitivity by almost ten-fold. Using an ITC200, we confirmed binding of the HIV-1 N-terminal capsid (CA^N) domain to CypA with micromolar affinity (5 μ M). Moreover, under identical buffer and temperature conditions, we also detected weak binding between the HIV-2 CA^N domain and CypA (Fig. 1a,b), with an affinity of 91 μ M. To demonstrate that this is a specific interaction, we repeated these experiments in the presence of cyclosporin, a competitive inhibitor of CypA. Notably, addition of 10 μ M cyclosporin blocked all binding (Fig. 1c,d). Thus, CypA has preexisting weak but specific affinity for HIV-2. As rhesus CypA is identical in sequence to human CypA, these results can be directly compared to previous studies on human CypA.

Two mutations increase affinity and retarget CypA to HIV-2

The CypA domain of RhTC has two coding mutations (D66N and R69H) with respect to the parental CypA gene. We examined why these two mutations have been selected by determining their effect on binding and cellular viral restriction. We have sought to avoid confusion by defining the residues at positions 66 and 69 such that parental CypA is denoted DR and the CypA borne by rhesus TRIMCyp (RhTC) is denoted NH. First, we compared binding of RhTC CypA (NH) and the individual CypA mutants D66N (NR) and R69H (DH) to HIV-1 and HIV-2 capsids (CA^N domain) by ITC. RhTC bound HIV-2 with an affinity of 3 μ M (Fig. 2) and showed no binding to HIV-1. Next, we tested the single mutation R69H (DH) and found that it broadened CypA binding specificity by maintaining micromolar

binding to HIV-1 CA^N and also conferring micromolar binding to HIV-2 CA^N (Fig. 2a). The second single mutation, D66N (NR), switched the binding specificity of CypA from HIV-1 CA^N to HIV-2 CA^N (Fig. 2a).

These binding experiments contradict published restriction data in which HIV-1 is restricted by NR but not by DH¹⁵. After discussing our findings, Virgen *et al.* found that NR and DH were mislabeled in their study; thus, their findings are in agreement with ours (P. Bieniasz, personal communication). To confirm the data, we independently repeated the restriction experiments. We expressed RhTC (NH), single mutants NR and DH and rhesus TRIMCyp bearing parental CypA (CypTC; DR) in permissive feline CRFK cells and tested the cells for their sensitivity to infection by the green fluorescent protein (GFP)-tagged viral vectors HIV-1 GFP and HIV-2 GFP, as described¹⁹, and plotted their infectious titers (Fig. 2b,e). As a control, we also measured infection sensitivity in unmodified CRFK cells.

HIV-1 GFP was not restricted by RhTC (NH) or the single mutant NR but was restricted by CypTC (DR) and the single mutant DH (Fig. 1b,d). Likewise, whereas CypTC (DR) restricts HIV-2 GFP, it is most strongly restricted by RhTC (NH) (Fig. 2c,e). These experiments confirm our binding data and demonstrate that binding and restriction broadly correlate: binding correlates with restriction, and a lack of binding correlates with permissive infectivity. We were not able to correlate the strength of binding with the strength of restriction, presumably because we measured monomeric binding rather than the predicted dimeric binding of the full-length protein. Furthermore, once binding is saturated—for example, by high local concentrations of TRIMCyp or virus—differences in affinity will be obscured. We found that HIV-1 did not bind, nor was it restricted by, a TRIMCyp with an asparagine at position 66, but binding and restriction were unaffected by whether arginine or histidine occupied position 69. This is in agreement with published structural data on the HIV-1–CypA interaction. In the solved HIV-1–CypA complex (PDB 1AK4), Arg69 makes no interactions that are essential in binding HIV-1, nor in stabilizing the CypA binding site²⁰. Also, mutation between arginine and histidine (in R69H (DH)) preserves the positive charge, whereas switching between aspartic acid and asparagine (in D66N (NR)) results in loss of a negative charge and is therefore considerably more destabilizing.

Structural basis for the HIV specificity of RhTC

Parental CypA had a micromolar affinity for HIV-1 and a weak affinity for HIV-2. In RhTC, this situation was reversed, such that HIV-2 bound with a micromolar affinity and HIV-1 binding was lost. This pattern of binding suggests that there are fundamental differences between the capsids (CA) of HIV-1 and HIV-2. We reasoned that, if these differences dictate viral sensitivity to restriction, they could be used to predict the restriction susceptibility of a particular lentivirus.

To address this question we solved the 1.2-Å resolution structure of the N-terminal domain of HIV-2 capsid (CA^N) and compared it to the previously solved structure of HIV-1 (Table 1). This is the first example of an HIV-2 capsid domain structure. Although there is sequence variation between HIV-1 and HIV-2, the overall structural topology is similar (C α atoms show an r.m.s. deviation of 2.4 Å; Fig. 3a). An extended exposed loop that connects helices 4 and 5 forms the CypA-binding site²¹. The sequence of this loop varies in 6 out of 14 residues between HIV-1 and HIV-2; yet, the conformation of the HIV-2 loop superposes closely with HIV-1, particularly with the NMR structure (PDB 1GWP)²², until around position CA 88 in HIV-1 (Fig. 3a and Supplementary Fig. 1). The loop conformations diverge at this point owing to a deletion in the CypA-binding loop of HIV-2. This deletion has previously been mapped to position CA 86 (refs. ^{17,23}); however, the structure clearly shows that it is equivalent to position CA 88. Notably, the structure of the ROD HIV-2 isolate analyzed here is typical of HIV-2 sequences with respect to the shorter loop sequence

(data not shown). The effect of the position CA 88 deletion is to substantially alter the loop conformation of HIV-2, resulting in a main chain displacement of around 4 Å with respect to HIV-1 (Fig. 3). As can be seen in the HIV-1–CypA complex, this part of the capsid loop is in direct contact with the loop from CypA containing residues 66 and 69 (Fig. 3b)²⁰. Thus, we hypothesised that the mutations at positions 66 and 69 in RhTC may have altered the structure of CypA such that it can discriminate between the different loop conformations of HIV-1 and HIV-2 that are mediated by position CA 88.

RhTC has a new active site conformation

To address the effects of RhTC mutations D66N and R69H, we solved the structure of its CypA domain to 1.5-Å resolution. The scaffold of RhTC CypA and parental CypA (PDB 2CPL)²⁴ superpose closely, with an r.m.s. deviation of 1.2 Å (Ca atoms). However, active site loop_{64–74} has undergone a complete rearrangement, leading to a >16 Å shift in the main chain (Fig. 4a). Mutation D66N disrupts a bifurcated hydrogen bond with the main chain nitrogen atoms of residues 73 and 74 that constrains the conformation of loop_{64–74} (Fig. 4b,c). The loss of these constraining interactions releases the loop, leading to a concerted restructuring of all ten loop residues. The new extended loop conformation in RhCyp forms a stable β-zipper structure that is held in place by main chain hydrogen bonds (Fig. 4c). The loop is not a product of crystal packing, and the electron density for this region is excellent, with a *B*-factor of ~13 Å² (Supplementary Fig. 2). Supplementary Movie 2 illustrates the magnitude of this change and shows how the structure has gone from a ‘closed fist’ to an ‘open palm’ conformation. This rearrangement has also propagated changes elsewhere in the structure, including in Arg55, the proposed catalytic residue for *cis-trans* isomerization.

Superposition of RhTC CypA on the HIV-1–CypA complex suggests why RhTC no longer binds HIV-1. The cyclophilin-binding loop of HIV-1 binds into a long groove or slot, with CA Pro90 at one end and CA Ala88 at the other (Fig. 4d). The rearrangement of loop_{64–74} in RhTC occurs in parallel with this groove and, despite the extensive changes, does not occlude it in any way (Fig. 4d,e). However, the direction of the rearrangement is directly away from HIV-1, disrupting all interactions with loop_{64–74}, including an interaction between CA Ala88 and CypA Gly72 (Fig. 4d,e and Supplementary Movie 2).

Next, we attempted to address why HIV-2 binds CypA weakly and how D66N and R69H increase affinity. As we were unable to grow complexed crystals of HIV-2 and RhTC, we took advantage of the high degree of structural homology between HIV-1 and HIV-2 to superpose HIV-2 onto the HIV-1–CypA complex. It has previously been shown that the binding loops of divergent O group viruses bind in the HIV-1–CypA complex active site groove, further validating this approach²⁵. From the superposition of HIV-2 on the complex, it is clear that, because of the deletion of CA Ala88, HIV-2 cannot form the same interactions with loop_{64–74} as HIV-1 does, suggesting why HIV-2 has a much lower affinity to parental CypA (Fig. 4f). To confirm this hypothesis, we introduced CA Ala88 into HIV-2 and found that addition of this residue was sufficient to confer an HIV-1–like affinity to parental CypA of ~8 μM (Supplementary Fig. 3). The open palm conformation present in RhTC CypA opens up the binding groove around the CA 88 deletion end of the HIV-2 cyclophilin binding loop, improving the surface complementarity (Fig. 4f,g). We did not, however, attempt to define the particular interactions that occur between HIV-2 and RhCyp, as the position of side chains may vary in the complex. Of the changes accompanying the rearrangement of loop_{64–74} in RhTC, it is worth noting that the movement of Arg55 places its guanidinium group in proximity with the ‘GP’ motif in HIV-2 (Fig. 4g). Arg55 is a key residue in the HIV-1–CypA interaction, where it is thought to facilitate catalysis by stabilizing a pyramidal *sp*³ hybridization state of the proline nitrogen atom in the transition state²⁵.

Residue 88 determines virus sensitivity to RhTC restriction

The HIV-2 and RhTC structures suggest that HIV-1 and HIV-2 have alternative loop conformations caused by a conserved deletion in position CA 88 and that these different loop conformations are differentiated by a new active site conformation in RhTC caused by the D66N and R69H mutations. To test this mechanism, we examined the cellular restriction of lentiviruses by RhTC in two ways. First, we investigated whether variation of position CA 88 alters sensitivity to restriction by RhTC. Second, we tested whether the restriction of naturally occurring lentiviruses can be predicted from the sequence at position CA 88.

Of the approximately 7,200 M group HIV-1 CA sequences available at the Los Alamos HIV sequence database, all but 15 (>99.8%) have an alanine at position CA 88. Conversely, O group HIV-1 viruses are far more variable at this position, with only 7 of 66 sequences encoding alanine at CA 88 (data not shown). We reasoned that, if position CA 88 is important in determining restriction, then M group and O group viruses may have different restriction sensitivities to RhTC. Replacing the M group gag sequence with the model O group HIV-1 capsid sequence derived from isolate MVP5180 (ref. ²⁶) conferred sensitivity to RhTC (Fig. 5a). MVP5180 has a methionine at position CA 88, a residue found in 10 out of 66 O group sequences. O group viruses have a methionine, valine, isoleucine or alanine at position CA 88, of which valine is the most common, found in 35 out of 66 sequences. To test whether variation in position CA 88 is sufficient to alter sensitivity to RhTC, we made a single A88V mutation in a HIV-1 M group virus. A88V conferred sensitivity to restriction. Mutation of CA Ala88 to other O group residues, such as the methionine found in MVP5180, similarly conferred restriction (data not shown) (Fig. 5b). To show that these changes are not context dependent, we made the reciprocal M88A mutant in O group MVP5180 and showed that this abolished restriction by RhTC (NH) and mutant NR, while maintaining restriction by DH and CypTC (DR) (Fig. 5c); thus, the M88A mutant behaved similarly to an M group HIV-1 (Fig. 2b,d).

Next, we tested whether the residue at position CA 88 in naturally occurring viral sequences predicts susceptibility to RhTC restriction. We analyzed the restriction profiles of chimeric HIV-1 GFP vectors bearing CA residues 79–98 (the CypA binding loop) from various naturally occurring HIV-1 O group viruses. The CA sequences were selected to represent the variation of O group viruses across this region. All the chimeras were restricted by RhTC except AY302647, and this virus uniquely encoded an alanine at position CA 88 (Fig. 5d,e). Thus, even in the context of variable O group CypA-binding loops the residue at position CA 88 dictates susceptibility to RhTC. In all of the restriction experiments, treatment of cells with 5 μ M cyclosporin rescued restricted infectivity, demonstrating that this is a specific effect (data not shown).

The structures, ITC binding and cellular restriction data all showed that the correlation between variation at position CA Ala88 in the capsid and restriction by RhTC is mediated by the residue at position 66. CypA containing Asn66 restricts lentiviruses, except those with an alanine at position CA 88, that is, HIV-2 and some O group, but not M group, viruses. CypA containing Asp66 restricts lentiviruses differently depending on whether they have a deletion at position CA 88; that is, it restricts HIV-1 M and O group viruses more potently than it restricts HIV-2 viruses. Thus, the ability of RhTC to restrict a particular lentivirus is strongly influenced by whether the side chain on a single residue is a carboxylate or a terminal amide.

CypA targets HIV-1 and HIV-2 using different mechanisms

As mutations at positions 66 and 69 have markedly different effects on HIV-1 binding and restriction, it is reasonable to suppose that they have different effects on the structure of

CypA and confer increased binding to HIV-2 in alternative ways. We decided to use ITC to compare the mechanism of binding of HIV-1 and HIV-2 to determine whether the mutants in RhTC increase binding to HIV-2 in different ways. The interaction of HIV-2 CA with RhTC (NH) and the DH and NR mutants was far less exothermic and was accompanied by a larger ΔS than the HIV-1 CA–CypA interaction (representative fits give $\Delta H = -1,156 \text{ cal mol}^{-1}$ and $\Delta S = 19.2 \text{ cal mol}^{-1} \text{ deg}^{-1}$ for HIV-2–RhTC compared to $\Delta H = -6,053 \text{ cal mol}^{-1}$ and $3.49 \text{ cal mol}^{-1} \text{ deg}^{-1}$ for HIV-1–CypA). This indicates that the RhTC interaction with HIV-2 CA is driven entropically, whereas interaction of CypA with HIV-1 CA is driven enthalpically. We confirmed these different binding mechanisms by ΔC_p analysis. Binding of CypA to HIV-1 CA occurred with a negative ΔC_p value of $-285 \text{ cal mol}^{-1} \text{ K}^{-1}$; conversely, the interaction between RhTC and HIV-2 CA had a positive ΔC_p ($+65 \text{ cal mol}^{-1} \text{ K}^{-1}$) (Fig. 6, inset). A positive ΔC_p is unusual in protein-protein recognition and may indicate that binding of RhTC to HIV-2 is accompanied by burial of polar interfaces or conformational stabilization.

Finally, we examined the types of interaction made during HIV-2–RhTC binding. Measuring interaction in buffers with different enthalpies of ionization revealed that the binding of HIV-2 to RhTC results in partial deprotonation (Fig. 6). We did not observe this binding-associated deprotonation in the NR mutant, suggesting that the histidine at position 69 in RhTC confers binding to HIV-2 CA by facilitating a charged interaction. This suggests that the two mutations at positions 66 and 69 operate independently and through different interactions. As we have shown, this results in their having different effects on overall lentiviral specificity. Mutation at position 69 confers broader specificity, whereas mutation at position 66 retargets to specific viruses. It is possible that there are other TRIMCyps that closely resemble RhTC but that either have broader lentiviral specificity or can restrict lentiviruses not normally targeted by CypA.

DISCUSSION

In a notable example of counterevolution between host and pathogen, CypA has been duplicated and fused to the antiretroviral protein TRIM5, allowing it to be freely targeted against viruses that recruit CypA. Here we have shown that CypA has preexisting weak affinity to HIV-2 that has been increased by two mutations in the CypA domain of RhTC, concomitantly switching its specificity from HIV-1, a virus derived from chimpanzees, to HIV-2, a member of a different viral lineage derived from sooty mangabeys^{27,28}. One of these mutations, R69H (DH), gives a broad-specificity TRIMCyp that can bind and restrict viruses from both lentiviral lineages. The second mutation, D66N (NR) completes the retargeting by making a potent HIV-2 restriction factor at the cost of the loss of HIV-1 specificity. We have further shown that the molecular mechanism by which RhTC differentiates between HIV-1 and HIV-2 involves detection of inherent differences in the loop conformations of HIV-1 and HIV-2 capsid that are in turn mediated by a single position in the capsid sequence (CA 88). Finally, correlated binding and restriction experiments show that the restriction sensitivity of naturally occurring viruses can be predicted on the basis of the sequence at this position.

Our results also reveal clear differences in the structural, thermodynamic and functional behavior of HIV-2 and the M and O group HIV-1 viruses. These differences are of interest given the relative success in the human population of the M group virus over the O group virus, which arose from an independent zoonotic event²⁸. Unlike the HIV-1 M group, the O group zoonosis has not led to pandemic levels of infection in humans, and these viruses may therefore be under stronger selection pressure, as evidenced by the greater variation²⁹ that we have demonstrated functionally in a part of the CA known to interact with the host via

CypA and TRIM5 α ^{8,13,30,31}. Whether TRIM5 α or CypA are responsible for this variation remains unclear.

TRIMCyp has provided a unique opportunity to investigate the molecular basis of lentiviral–CypA specificity in a context in which the biological role of the interaction is clear. This has led to our unexpected discovery that not only does CypA have a broader specificity for lentiviruses than previously thought but that both CypA and lentiviruses are thermodynamically poised to switch their interaction on or off with a single mutation. These results provide evidence for the Red Queen hypothesis³², which proposes that host and pathogen are under constant selective pressure from each other to evolve and gain the advantage. We cannot know the identity of the virus behind the selection of rhesus TRIMCyp, but it has left its evolutionary and molecular footprint in the CypA active site. We have shown that the success of RhTC against viruses extant today is dependent on how well they fit that footprint. Notably, we have also shown that RhTC targets conserved differences in the viral capsid, suggesting that viruses too have been subject to selective pressure at these positions.

It is interesting to note that, in the case of the antagonistic relationship between lentivirus and TRIMCyp, as well as TRIM5 α , small changes in either the viral CA sequence or the CypA (or PRYSPRY) virus-binding domain dictate restriction or replication^{4,15,31,33}. There seems to be a similar delicate balance between the restriction factor APOBEC3G and its viral antagonist Vif. Point mutations in APOBEC3G affect its species-specific antiviral specificity via its sensitivity to the viral Vif protein³⁴. It therefore seems that, at least for retroviruses, the balance between restriction and replication is finely balanced.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/nsmb/>.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was funded by the UK Medical Research Council and a Wellcome Trust fellowship (WT076608) to G.J.T. We would like to thank the European Synchrotron Radiation Facility (ESRF) staff at beamline ID14-1 and local contact E. Fioravanti. We also thank S. Hue, P. Bieniasz and J. Heeney for helpful discussion and A. Lever, D. Trono and A. Thrasher and L. Gurtler via the National Institute of Biological Standards and Controls (NIBSC) AIDS Reagents Programme, for reagents.

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Figure 1.

HIV-2 binds CypA with weak affinity. **(a,b)** Isothermal titration calorimetry (ITC) of parental CypA against the CA^N domains of HIV-1 **(a)** and HIV-2 **(b)**. Purified CA^N was titrated against CypA, and the equilibrium dissociation constant (K_d) was derived from the resulting binding isotherms. **(c,d)** Titrations were repeated in the presence of CypA inhibitor, cyclosporin (Cs). Addition of $10 \mu\text{M}$ Cs blocked binding of CypA to both HIV-1 **(c)** and HIV-2 **(d)**.

Figure 2.

Two CypA mutations dictate lentiviral binding and restriction sensitivity. **(a)** Equilibrium dissociation constants (K_d) of CypA–capsid interactions calculated by ITC and plotted on a log scale. Where binding was not detected, no bar is shown. Black bars denote binding to HIV-1 and gray bars denote binding to HIV-2. **(b–e)** Lentiviral vectors derived from HIV-1 or HIV-2 and encoding GFP were titrated onto unmodified CRFK cells (C) or CRFK cells expressing rhesus TRIMCyp (RhTC) or the mutants, as shown. Titers are plotted as infectious units per ng of reverse transcriptase activity and are representative of infections performed with two virus preps. Errors bars show s.e.m. of titers determined at three doses of virus. In **b,c**, black bars represent RhTC (NH) and gray bars represent CypA-encoding TRIMCyp (DR). In **d,e**, Black bars represent mutant R69H (DH) whilst gray bars represent D66N (NR).

Figure 3.

Structure of the N-terminal capsid domain of HIV-2. **(a)** The structure of the HIV-2 N-terminal capsid domain (CA^N) (yellow) was superposed on the structure of HIV-1 (PDB 1GWP)²², shown in gray. The gray sphere indicates the position of CA Ala88 in HIV-1, which is deleted in HIV-2. The extra residue in HIV-1 creates a kink in the cyclophilin-binding loop. **(b)** Structure of the HIV-1-CypA complex (PDB 1AK4)²⁰ in which HIV-1 is in gray and CypA is in green. The proximity of position CA Ala88 to the two mutations D66N and Arg69 (spheres) in CypA is shown.

Figure 4.

RhTC CypA has been re-targeted from HIV-1 to HIV-2. **(a)** The structure of the CypA domain from RhTC (yellow) is superposed on the CypA domain from (PDB 2CPL)²⁴ (gray). Loop_{64–74}, which contains the D66N and R69H mutations, has undergone a >16-Å rearrangement. **(b)** Loop_{64–74} in CypA is held in place by a bifurcated hydrogen bond between Asp66 and main chain nitrogen atoms from Thr73 and Gly74. **(c)** In RhTC CypA, loop_{64–74} forms a completely unrelated extended conformation that is stabilized by several main chain β-zipper interactions and by His69. **(d–g)** The active site of CypA or RhTC is shown as a gray molecular surface, with catalytic residue Arg55 and loop_{64–74} in yellow. The core residues of the cyclophilin-binding loops of HIV-1 (green) and HIV-2 (cyan) are shown. **(d)** HIV-1–CypA²⁰ complex showing hydrogen bond from CA Ala88 to loop_{64–74}. **(e)** RhCyp has been superposed on and replaces CypA. RhCyp loop_{64–74} has moved 16 Å away from HIV-1. **(f)** HIV-2 superposed on and replacing HIV-1 in the HIV1–CypA complex. Without CA Ala88, HIV-2 cannot hydrogen bond to loop_{64–74}. **(g)** RhCyp superposed on and replacing CypA from **c**. RhCyp loop_{64–74} movement improves complementarity with HIV-2. Arg55 has also moved into proximity with the GP motif of HIV-2.

Figure 5.

Capsid residue 88 is a key determinant of lentiviral sensitivity to RhTC. (**a–d**) Lentiviral vectors encoding GFP and derived from HIV-1 bearing an O group gag from MVP5180 (**a**), or HIV-1 CA mutant A88V (**b**), or MVP5180 gag mutant CA M88A (**c**), or HIV-1 bearing residues 79–98 of various O group HIV-1 isolates (**d**), were titrated onto unmodified CRFK cells (C) or CRFK cells expressing rhesus TRIMCyp (RhTC) or mutants thereof, and infectious titers were determined. Titers are plotted as infectious units per ng reverse transcriptase; error bars show s.e.m. of titers determined at three infectious doses and are representative of experiments performed with two independent virus preps. In **a–c**, RhTC is denoted by a black bar and the mutants by gray bars. In **d**, white bars denote infection in the presence of 5 μ M cyclosporin, and black bars denote infection without cyclosporin. (**e**) Alignment of O group CA residues 79–98 indicate that the only amino acid unique to the unrestricted chimera is an alanine at position CA 88, underlined. Similarity is shown: an asterisk indicates an identical residue, a period indicates a semiconserved substitution and a gap indicates no conservation.

Figure 6.

CypA targets HIV-1 and HIV-2 using different mechanisms. Deprotonation coupling in HIV-2–RhTC interaction. ITC experiments were carried out using a range of buffers with different ionization enthalpies, as described in Online Methods. Fitting to the equation $\Delta H_{\text{obs}} = \Delta H_{\text{bind}} + n_{\text{H}} \Delta H_{\text{ion}}$ (where ΔH_{obs} is the measured enthalpy, ΔH_{bind} is the reaction enthalpy and n_{H} the number of protons absorbed or released upon binding) indicates that binding of RhTC (NH) to HIV-2 is accompanied by partial deprotonation of a single group, whereas there is negligible change in protonation when HIV-2 binds mutant NR. Inset, $\Delta C(p)$ analysis of the interaction between HIV-1 and parental CypA and HIV-2 and RhTC. Binding experiments were carried out in the same buffer but at different temperatures and ΔH (cal mol⁻¹) plotted against temperature (K). Linear fits gave a negative $\Delta C(p)$ of -285 cal mol⁻¹ K⁻¹ for HIV-1 and a positive $\Delta C(p)$ of 65 cal mol⁻¹ K⁻¹ for HIV-2.

Table 1

Data collection and refinement statistics

	HIV-2	RhTC CypA
Data collection		
Space group	$P2_12_12$	$P2_12_12_1$
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	95.52 47.81 88.57	35.88 51.12 75.18
Resolution (Å)	42–1.2	42–1.5
R_{sym}	0.050(0.638)	0.079(0.196)
$I/\sigma I$	12.9(1.9)	11.2(4.9)
Completeness (%)	99.9(99.9)	96.5(86.4)
Redundancy	4.4(3.9)	3.6(3.5)
Refinement		
Resolution (Å)	1.2	1.5
No. reflections	113,327	23,059
$R_{\text{work}}/R_{\text{free}}$	0.17/0.22	0.16/0.21
No. atoms		
Protein	4,860	2,655
Water	406	253
<i>B</i> -factors		
Protein	18.4	11.5
Water	37.5	22.3
R.m.s deviations		
Bond lengths (Å)	0.018	0.018
Bond angles (°)	2.4	2.2

* Values in parentheses are for highest-resolution shell.