



The capsid protein of human immunodeficiency virus: interactions of HIV-1 capsid with host protein factors

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HIV-1 is a retrovirus that causes AIDS in humans. The RNA genome of the virus encodes a Gag polyprotein, which is further processed into matrix, capsid and nucleocapsid proteins. These proteins play a significant role at several steps in the viral life cycle. In addition, various stages of assembly, infection and replication of the virus involve necessary interactions with a large number of supplementary proteins/cofactors within the infected host cell. This minireview focuses on the proteomics of the capsid protein, its influence on the packaging of nonviral molecules into HIV-1 virions and the subsequent role of the molecules themselves. These interactions and their characterization present novel frontiers for the design and advancement of antiviral therapeutics.

Introduction

On the entry of HIV-1 into the cytoplasm of the host cell, retroviral single-stranded RNA is reverse transcribed into double-stranded DNA, which is translocated into the nucleus for integration into the host DNA. Transcription of viral DNA yields two large viral proteins, Gag (55 kDa) and GagPol (160 kDa), which interact with each other during viral assembly [1–4]. Gag consists of three major proteins, matrix (MA), capsid (CA) and nucleocapsid (NC), each of which play a significant role in the internal structural organization of viral particles. In addition, a p6 domain and two spacer peptides, p2 and p1, are also

present within Gag. The Pol domain of GagPol additionally is comprised of the reverse transcriptase, protease and integrase proteins.

During viral assembly, intact Gag proteins attach to the inner cell membrane via the myristoylated N-terminus of MA. Immature, non-infectious virions bud and are released from the host cell concomitant with the initial stages of maturation. Viral protease autocatalyzes its release from GagPol followed by processing of Gag and GagPol into their constituent mature proteins. The core of the virion includes a characteristic shell structure formed by mature CA proteins and

Abbreviations

aaRSs, aminoacyl-tRNA synthetases; CA, capsid protein; CA-CTD, C-terminal domain of CA; CA-NTD, N-terminal domain of CA; CyPA, cyclophilin A; CyPs, cyclophilins; hTRIM5 α , human TRIM5 α ; LysRS, lysyl-tRNA synthetase; MA, matrix protein; MHR, major homology region; MLV, murine leukemia virus; NC, nucleocapsid protein; rhTRIM5 α , TRIM5 α from rhesus macaque monkeys; TRIM5 α , tripartite motif 5 isoform alpha; VLP, virus-like particle.

contains the viral RNA genome coated by the NC protein. The mature virion can then infect other host cells. Along each step of the viral life cycle, viral RNA and proteins encounter as many as 250 host cell factors that either facilitate or restrict viral infection [5].

Lysyl-tRNA synthetase and tRNA^{Lys3}

HIV-1 reverse transcriptase catalyzes the synthesis of viral DNA using host cell tRNA^{Lys} as a primer for initiation. Synthesis of cDNA initiates from a primer binding site of 18 bases near the 5' end of viral genomic RNA. The 3' terminal end of tRNA is complementary to nucleotides in the primer binding site, although other regions in the viral RNA may also interact with the tRNA [6,7]. Although three human tRNA^{Lys} isoacceptors are selectively packaged into HIV-1 virions during assembly, only tRNA^{Lys3} is used as the primer for reverse transcription [7]. Different primer tRNAs are used by different retroviral families (e.g. tRNA^{Trp} for alpharetroviruses and tRNA^{Pro} for most gammaretroviruses) [8]. Other lentiviruses such as feline immunodeficiency virus, equine infectious anemia virus, simian immunodeficiency virus and HIV-2 use tRNA^{Lys3} as a primer. Human lysyl-tRNA synthetase (LysRS), a tRNA^{Lys}-binding protein responsible for aminoacylation of all three tRNA^{Lys} isoacceptors, is also packaged into newly formed HIV-1 virions [9]. The absence of other aminoacyl-tRNA synthetases (aaRSs) suggests that packaging is specific to LysRS [9,10]. LysRS directly interacts with Gag *in vitro* and can be packaged into virus-like particles (VLPs) composed only of Gag, independent of tRNA^{Lys3} or Gag-Pol [9]. Therefore, the current hypothesis for tRNA^{Lys} packaging involves an interaction between a Gag/Gag-Pol complex and LysRS/tRNA^{Lys3} complex.

Analyses of tRNA^{Lys3} anticodon mutants revealed a direct correlation between their ability to be incorporated into virions and their ability to undergo aminoacylation [11]. Because the aminoacylation defect of these tRNA variants was primarily in the K_m parameter, it was suggested that binding to LysRS rather than aminoacylation *per se* is a pre-requisite to packaging. This conclusion was subsequently verified in a separate study showing that LysRS mutants that lacked aminoacylation activity were still packaged into HIV particles, which also contained wild-type levels of tRNA^{Lys} primer [12]. Overexpression of exogenous wild-type LysRS in cells results in a two-fold increase in the uptake of both LysRS and tRNA^{Lys} into virions [13]. Interestingly, an N-terminally truncated LysRS variant ($\Delta N65$) with approximately 100-fold weaker affinity

for tRNA^{Lys} showed a slight increase in incorporation into virions compared to wild-type LysRS, possibly as a result of the higher amounts present in the cytoplasm [12]. However, virion tRNA^{Lys} levels displayed a slight decrease [12]. Taken together, these data show that binding to LysRS is critical for tRNA^{Lys} packaging into HIV, whereas aminoacylation is not. In addition, LysRS packaging is independent of tRNA packaging.

Although aaRSs cognate to the primer tRNA are strong candidates for packaging signals, the selective packaging of the aaRS itself differs among retroviruses [14]. Cen *et al.* [14] probed western blots of viral and cell lysates for the presence of LysRS, TrpRS and ProRS, cognate to primer tRNAs in HIV-1, Rous sarcoma virus and murine leukemia virus (MLV), respectively. Although, LysRS was detected in HIV-1 and TrpRS was seen in Rous sarcoma virus viral lysates, ProRS was not detected in MLV, suggesting that ProRS may not be a packaging signal for tRNA^{Pro} [14]. Gabor *et al.* [13] showed that overexpression of exogenous tRNA^{Lys3} resulted in higher incorporation into virions, increased tRNA annealing to viral RNA and greater infectivity of the virus. The absence of an accompanying increase in GagPol/Gag levels indicates that LysRS may be the limiting factor for tRNA^{Lys3} packaging [13]. Moreover, using small interfering RNA to silence LysRS mRNA causes an 80% decrease in newly synthesized LysRS in the cellular pool and a corresponding decrease in viral LysRS [15]. Viral tRNA^{Lys} isoacceptor levels reduce to approximately 40–50% of wild-type levels and a similar decrease in tRNA^{Lys} annealing and viral infectivity is also observed [15].

Human LysRS is member of the class II aaRS family. It is believed to function as a homodimer, with each monomer consisting of an N-terminal anticodon binding domain, a dimerization domain formed by motif 1, and motifs 2 and 3 that together constitute the aminoacylation active site (Fig. 1A,B). LysRS is one of nine aaRSs in the high molecular weight multi-synthetase complex observed in higher eukaryotic cells. A recently solved X-ray crystal structure of a tetrameric form of human LysRS provided insight into possible interactions with other proteins that comprise the multi-synthetase complex [16].

Based on the finding that VLPs composed only of HIV Gag protein package human LysRS, it was hypothesized that interactions between Gag and LysRS dictate LysRS packaging. An interaction between the proteins was confirmed by *in vitro* glutathione *S*-transferase pull-down studies using wild-type LysRS and truncated LysRS mutants, followed by testing their ability to be packaged into Gag VLPs *in vivo* [17]. Similar experiments with truncated Gag con-

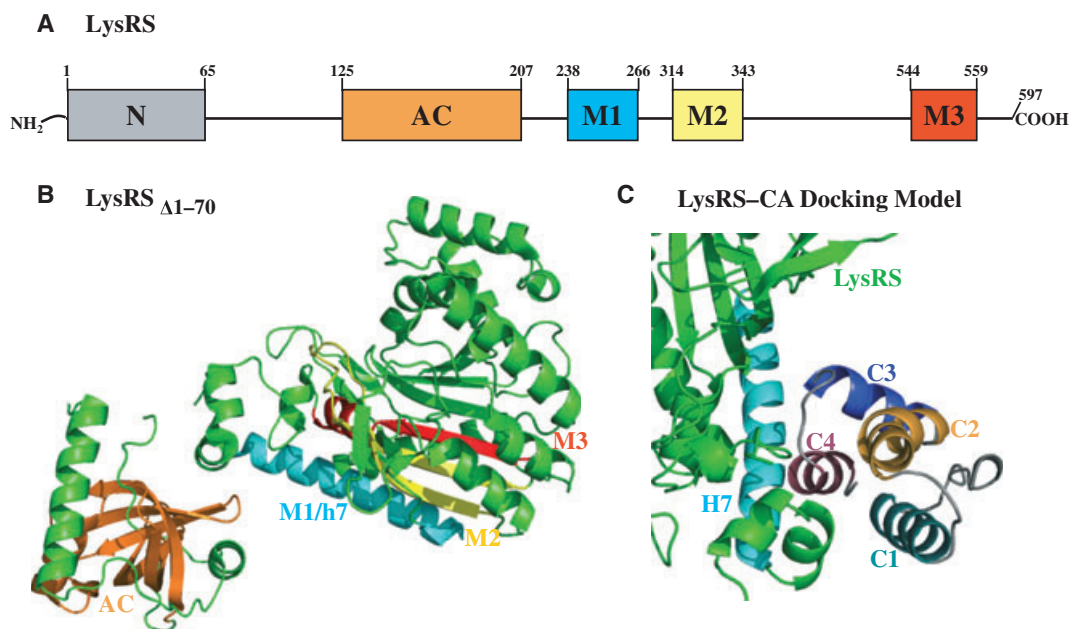


Fig. 1. Domains of human lysyl-tRNA synthetase. (A) Domain arrangement of LysRS. Amino acid positions of the N-terminal (grey), anticodon binding (AC, orange) and aminoacylation domain with characteristic class II aaRS sequence motifs 1 (cyan), 2 (yellow) and 3 (red) are shown. Motif 1 is part of the dimerization interface and motifs 2 and 3 form the aminoacylation active site. (B) Crystal structure of human LysRS monomer (Protein Data Bank code: 3BJU) with the first 70 amino acids deleted [16]. The anticodon domain and motifs 1, 2 and 3 are highlighted as in (A). (C) Computational docking model displaying the predicted LysRS–CA interaction. Kovalski *et al.* [23] proposed that helix 7 of LysRS (H7, cyan) binds helix 4 of CA-CTD (C4, magenta). Adjacent helices of CA (C1 in teal, C2 in orange, and C3 in blue) are also indicated.

structs localized sites of interaction in Gag and LysRS to residues 308–362 at the C-terminal end of CA and 208–259 of the LysRS motif 1 [17]. Interestingly, both regions are critical for formation of the homodimer interfaces within each protein. Site-directed mutants that disrupt homo-dimerization of either LysRS (alanine substitutions at residues R247, E265 and F283) or CA (alanine substitutions at residues W317 and M318; residues are numbered from the beginning of Gag; Fig. 2A) have no significant effect on the Gag–LysRS interaction, possibly as a result of the formation of a heterodimeric Gag–LysRS complex [18]. Gel chromatography binding studies are consistent with heterodimer formation and an equilibrium binding constant of 310 ± 80 nM was determined for the Gag–LysRS complex using fluorescence anisotropy [18]. A comparison of X-ray crystal structure data suggests that the interaction domain of CA can adopt different dimerization interfaces by swapping the major homology region (MHR) element between monomers [19,20]. The MHR, part of helix 1 in the C-terminal domain of CA (CA-CTD), is a highly conserved domain present in all retroviral CA proteins [21]. Plasticity would be advantageous to the various interactions where CA plays a role [22].

Fluorescence anisotropy binding measurements revealed that LysRS missing the N-terminal 219 residues retains a high affinity to CA, and that the CA-CTD is sufficient to bind LysRS [23]. Using NMR spectroscopy, chemical shift perturbations of residues in and around helix 4 ($^{211}\text{LEEMMT}^{216}$) of CA-CTD were observed upon LysRS binding. Residues T210, M214 and M215, along with a nearby H226, were implicated as critical by peptide binding studies and alanine scanning mutagenesis [23]. Computational docking and biochemical data support a direct interaction between helix 7 of LysRS and helix 4 (C4) of CA-CTD (Fig. 1C) [23]. Screening of small molecules, synthetic peptides and nucleic acids, which block the Gag–LysRS interaction with minimal toxicity to the host cell, is being explored as a strategy to inhibit HIV-1 replication.

Cyclophilin A

Cyclophilin A (CyPA) is a peptidyl–prolyl *cis–trans* isomerase and a member of the cyclophilin (CyP) family. These proteins localize to different cellular compartments in various organisms [24–26]. CyPA catalyzes a peptidyl–prolyl *cis–trans* isomerization

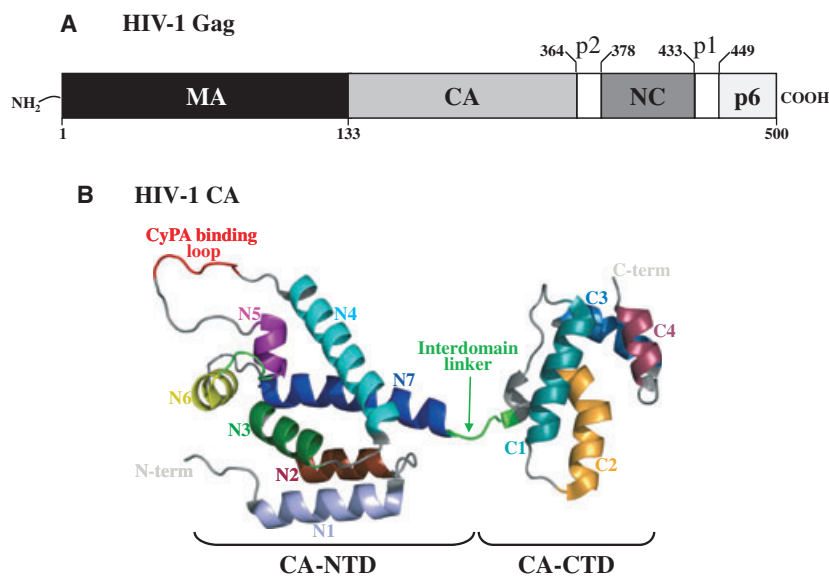


Fig. 2. HIV-1 Gag domains and CA crystal structure. (A) Domain arrangement of Gag. Different protein domains that comprise Gag are shown with their residue numbers: matrix (MA, black), capsid (CA, grey), nucleocapsid (NC, dark grey), spacer peptides p1 and p2 (white) and p6 (light grey). (B) X-ray crystal structure of HIV-1 CA (Protein Data Bank code: 1E6J). Helices N1–N7 in the CA-NTD and C1–C4 in the CA-CTD are indicated [79]. The CyPA-binding loop (red) and interdomain linker (green) are highlighted.

reaction, to generate the correct conformation of proline, which is a rate-limiting step in protein folding. This ability possibly influences the role of these enzymes in signaling, RNA splicing, gene expression and protein trafficking in cells [25,27]. CyPs are targeted by the immunosuppressive drug cyclosporin A (CsA), which inhibits peptidyl-prolyl isomerase activity and disrupts protein folding [28,29]. Over a decade ago, interactions between HIV-1 Gag and human CyPs A and B were identified using a yeast two-hybrid screen, but only the CyPA interaction was detected *in vivo* [30,31]. More specifically, CyPA binds an exposed proline-rich loop in the HIV-1 N-terminal domain of CA (CA-NTD) and is incorporated into HIV-1 virions at a concentration of approximately 200 molecules of CyPA per virion [30,32]. VLPs lacking CyPA possess normal morphology and can penetrate host cells, but are defective in the reverse transcription of viral RNA [33–35]. Furthermore, CsA prevents the incorporation of CyPA into virions, resulting in reduced infectivity. The necessity of CyPA for viral infectivity makes it a potential therapeutic target.

CyPA is shaped like a β -barrel formed by eight anti-parallel beta strands with two alpha helices that cap the top and bottom of the barrel (Fig. 3) [36,37]. The active site, in a hydrophobic pocket on the protein surface, is the binding site of CsA and its analogs. Mutational analyses and co-crystallization data have isolated residues $^{87}\text{HAGPIA}^{92}$ in CA, nicknamed the cyclophilin binding loop, as the specific binding site for CyPA [30,32,38–40] (Figs 2B and 3). Two other binding sites on CA, specifically GP^{157} and GP^{224} , with higher affinities than the GP^{90} site within the cyclophi-

lin binding loop, have also been identified [41]. A glycine-proline motif appears to be a prerequisite for binding the CyPA active site. The specific CA residue P90 is critical for the CyPA–CA interaction, and CyPA may also act as a molecular chaperone to ensure proper folding of CA [42,43]. The highly exposed, flexible cyclophilin binding loop lies in the CyPA binding pocket proximal to other CA and CyPA atoms that stabilize binding through hydrophobic interactions [38]. A hydrogen bond formed between R55 of CyPA and P90 of CA anchors the proline, whereas the oxygen atom of G89 rotates from *cis* to *trans* (Fig. 3) [44]. Other active site residues include H54, N71, N102, H126 and W121, with all except the latter being critical for virion incorporation of CyPA.

Endrich *et al.* [41] reported a higher affinity of CyPA for mature CA ($K_D \sim 0.6 \mu\text{M}$) compared to Gag (K_D , $8.2 \mu\text{M}$) using fluorescence studies, whereas Bristow *et al.* [45] observed contradictory results using an ELISA. Interestingly, both Gag and CA employ different steric conformations of the cyclophilin binding loop to bind CyPA. A critical hydrogen bond is required between W121 of CyPA and I91 of mature CA for stability of the CyPA–CA complex, although this interaction is not required by Gag [46]. This suggests that the CyPA loop undergoes a refolding event after maturation of Gag, and proteolytic processing of Gag when bound to CyPA prevents this conformational switch [46].

Colgan *et al.* [47] used a yeast two-hybrid system and glutathione *S*-transferase pull-down assays to test the ability of Gag mutants to bind CyPA and to self-associate. They observed that mutants unable to

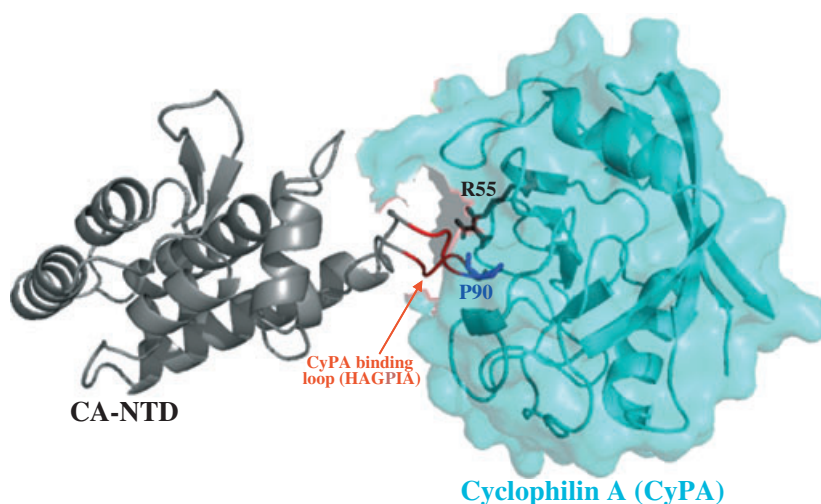


Fig. 3. The CA–CyPA complex. The $^{87}\text{HAGPIA}^{92}$ sequence of CA-NTD (grey) is displayed in red with Pro90 highlighted in blue. The active site residue Arg55 (black) in a hydrophobic pocket on the surface of CyPA (cyan) is also highlighted.

multimerize were deficient in CyPA binding. CyPA binding to mature CA causes structural changes in the CA-CTD, as is evident by the inability to form a C198–C218 disulfide bond, which is surprising considering that $^{87}\text{HAGPIA}^{92}$ is located in the CA-NTD [46]. The latter result suggests an effect of CyPA on CA-CTD function because the CA-CTD sequences required for dimerization may undergo a conformational change with a functional consequence on viral infectivity. Overall, the ability of CyPA to bind both Gag and CA presents the possibility of two such populations within virions. Consistent with this hypothesis, the structural change in the CTD dimerization motif caused by CyPA could be instrumental in destabilization of the CA core during or prior to reverse transcription within the host cell [38]. To that effect, the ability of CA to form dimers and oligomers *in vitro* was severely diminished in the presence of CyPA [48].

The exact role of CyPA in the viral lifecycle is unclear and remains a subject of intense debate [49–51]. The significance of this interaction is supported by an alignment of primate lentiviruses, which showed high conservation of the CyPA binding loop on the outer surface, in addition to GP motifs [52], indicating that recruitment of CyPA by HIV-1 is crucial. Both of these conserved elements are also found in equine infectious anemia virus and feline immunodeficiency virus. Thus, the use of the characterized CA–CyPA interaction as a tool to effectively inhibit HIV-1 replication comprises another approach that is being developed in the fight against AIDS. Liu *et al.* [53] designed two antisense RNAs that significantly impair HIV-1 replication: a modified derivative of U7 small nuclear RNA that interferes with CyPA splicing, and a small hairpin RNA that targets two different coding regions of CyPA. A number of synthesized thiourea derivatives

that possess dual activity against both CyPA and CA are currently undergoing *in vivo* characterization [54].

Tripartite motif (TRIM) proteins

Host cell restriction factors have evolved along with retroviruses to provide an innate immune response that inhibits retroviral infection. One such factor is tripartite motif 5 isoform α (TRIM5 α) which is virus- and species-specific in primates [55]. First identified by Stremlau *et al.* [56] using a genetic screen, TRIM5 α from the rhesus macaque monkeys (rhTRIM5 α) was found to restrict HIV-1 infection. As examples of the species-specificity of these proteins, human TRIM5 α (hTRIM5 α) inhibits N-tropic MLV (N-MLV) in human cells [57,58], but only weakly blocks HIV-1; rhTRIM5 α blocks simian immunodeficiency viruses from tantalus monkeys but not that from the rhesus macaque. Moreover, the amino acid sequence of hTRIM5 α is 87% identical to that of rhTRIM5 α . Restriction factors such as Ref1 in humans, which target N-MLV and lentivirus susceptibility factor 1 in rhesus monkeys and which restrict a broad array of viruses, including N-MLV, HIV-1 and HIV-2, were found to be species-specific variants of TRIM5 α [57–59].

TRIM5 α is a member of a large family of tripartite motif proteins with diverse functions that localize to different cellular compartments [60]. The retroviral CA protein determines susceptibility to a particular TRIM5 α , and it is proposed that TRIM5 α targets and binds the incoming viral capsid upon entry into the host cell (Fig. 4A). TRIMs are also known as RBCC proteins because they contain RING, B-box 2 and coiled-coil domains [60]. In addition, TRIM5 α is the only TRIM member with a PRYSPRY domain (Fig. 4B), as also found in members of the immunoglobulin family,

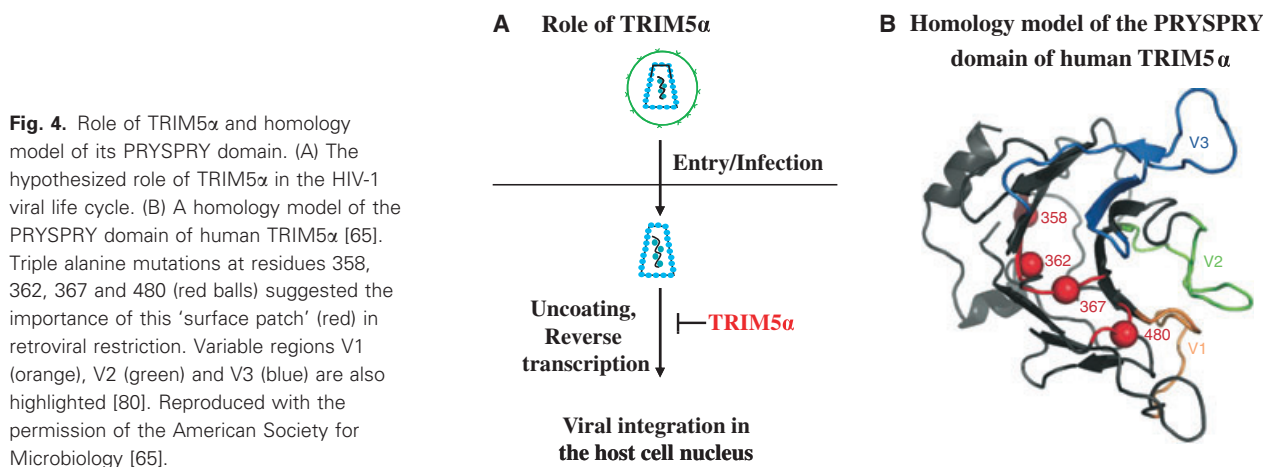


Fig. 4. Role of TRIM5 α and homology model of its PRYSPRY domain. (A) The hypothesized role of TRIM5 α in the HIV-1 viral life cycle. (B) A homology model of the PRYSPRY domain of human TRIM5 α [65]. Triple alanine mutations at residues 358, 362, 367 and 480 (red balls) suggested the importance of this 'surface patch' (red) in retroviral restriction. Variable regions V1 (orange), V2 (green) and V3 (blue) are also highlighted [80]. Reproduced with the permission of the American Society for Microbiology [65].

indicating the importance of this domain in restriction. Mutational and chimeric analyses of TRIM5 α concluded that the region between residues 320–345 of the PRYSPRY domain was important for restriction, but residues in the coiled-coil domain were particularly important for N-MLV inhibition [61–63]. Interestingly, mutation of hTRIM5 α residue R332 to proline (i.e. the corresponding residue in rhTRIM5 α) enables restriction of HIV-1, but to levels lower than that displayed by wild-type rhTRIM5 α , and a P332R mutation in rhTRIM5 α only strengthens inhibition of HIV-1 [63,64]. More recently, Sebastian *et al.* [65] created a homology model of the PRYSPRY domain of human TRIM5 α using the known structures of the same domain from three other proteins [66–68]. Furthermore, these authors showed that alanine mutations at clusters of surface residues of TRIM5 α reduced restriction activity against N-MLV CA but retained their binding ability (Fig. 4B) [65].

Stremlau *et al.* [69] developed a novel sucrose gradient centrifugation assay to separate cytosolic soluble CA proteins and particulate capsids. Using this assay and western blots to detect specific CA proteins, they showed that expression of hTRIM5 α in target cells caused a decrease in the amount of particulate N-MLV capsids and a concomitant increase in cytosolic N-MLV CA protein, whereas the expression of rhTRIM5 α decreased the particulate HIV-1 capsids [69]. Simultaneous increase in HIV-1 CA protein in the cytosolic fraction was not detected, possibly because the increase was minimal. This supports the hypothesis that TRIM5 α causes premature uncoating/disassembly of the viral capsid, which is detrimental to reverse transcription [70] and suggests an interaction between CA and TRIM5 α multimers in the intact virion core (Fig. 4A). A model for the organization of the viral

core proposes that the conical capsid shell forms a curved lattice containing cages of hexameric CA rings with the narrow and wide ends of the cone allowed to close through pentagonal defects [71]. TRIM5 α has been shown to oligomerize into trimers, suggesting two possible binding sites with CA: one in the center of the hexameric ring and another in a trilobed hole flanked by the hexamer spokes [72].

Although the mechanism of restriction is still unclear, reverse transcription of the viral RNA is inhibited. Stability of TRIM5 α factors decreases when they come in contact with a restriction-sensitive retroviral core [73]. For example, host cells exposed to HIV-1 resulted in the destabilization of rhTRIM5 α but not hTRIM5 α and restriction-sensitive N-MLV alters the stability of hTRIM5 α , which is unaffected by restriction-insensitive B-MLV [73]. TRIM5 α is ubiquitinated in cells and is rapidly turned over by the proteasome. The absence of destabilization in the presence of protease inhibitors implies that TRIM5 α factors are targeted for degradation once they interact with a restriction-sensitive retroviral core [73]. However, the presence of protease inhibitors does not rescue infectivity, indicating that interaction with TRIM5 α renders the CA core inactive, possibly by disrupting the arrangement of CA molecules forming the core. Alternatively, proteasomal degradation of the TRIM5 α –CA complex could lead to disassembly of the CA core and premature uncoating. Rhesus monkey TRIM5 α also appears to inhibit assembly prior to budding in a mechanism distinct from post-entry restriction (i.e. by rapid degradation of Gag polyprotein) [74].

A novel fusion protein between TRIM5 α and CyPA, found only in owl monkeys, has recently been identified [75]. Although the CA–CyPA interaction is required for HIV-1 infectivity (see above), the same interaction

is found to be inhibitory in owl monkeys. Mutations that block this interaction lower HIV-1 infectivity in human cells but rescue infectivity in owl monkey cells [76], and silencing CyPA expression was also found to rescue infectivity [75]. In the TRIM–CyPA protein, CyPA replaces the PRYSPRY domain, but maintains the same function because the CyPA domain of TRIM5 α –CyPA binds HIV-1 CA *in vitro* [77].

The restriction activity of rhTRIM5 α makes this protein a potential treatment against AIDS. The design of both small molecules such as mini rhTRIM5 α with the minimum required domains for antiviral activity or molecules with the ability to induce a conformational change of hTRIM5 α to mimic rhTRIM5 α comprise attractive strategies [78].

Conclusions

HIV-1 CA plays an important role in structural assembly and organization of the virion and indirectly in infectivity. Although the CA-NTD and CA-CTD are connected by an interdomain linker that allows for independent domain flexibility, solvent exposed regions such as the CyPA-binding loop extend the capability for functional interactions with partner proteins (Fig. 2B). The dimerization motif in CA-CTD encompasses the conserved MHR, which is essential for viral assembly. Indeed, domain-swapping dimerization results in the MHR at the dimer interface with several residues required for stability of the dimer [19]. This plastic architecture of the CA-CTD dimer possibly aids in the rapid assembly/disassembly of the retroviral capsid during the viral cycle. In addition, interactions between CA and several key host protein factors suggest a more extensive role during key viral events. Cyclophilin and LysRS interact with different CA domains. TRIM5 α appears to facilitate capsid uncoating, the premature occurrence of which is detrimental to reverse transcription. Consequently, CA presents an attractive target for antiviral drug development. Therapeutics involving the simultaneous disruption of a key interaction and the perturbation of flexibility of CA may be the most effective against HIV-1. The molecular details of LysRS, CyPA and TRIM5 α interactions with CA remain incomplete and additional biochemical and biophysical studies will be necessary before the full structural and functional consequences of these interactions are understood.

Acknowledgements

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