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TRIM5 structure, HIV-1 capsid recognition, and innate immune signaling

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Abstract

TRIM5 is a restriction factor that blocks retrovirus infection soon after the virion core enters the cell cytoplasm. Restriction activity is targeted to the virion core via recognition of the capsid protein lattice that encases the viral genomic RNA. In common with all of the many TRIM family members, TRIM5 has RING, B-box, and coiled-coil domains. As an E3 ubiquitin ligase TRIM5 cooperates with the heterodimeric E2, UBC13/UEV1A, to activate the TAK1 (MAP3K7) kinase, NF- κ B and AP-1 signaling, and the transcription of inflammatory cytokines and chemokines. TAK1, UBC13, and UEV1A all contribute to TRIM5-mediated retrovirus restriction activity. Interaction of the carboxy-terminal PRYSPRY or cyclophilin domains of TRIM5 with the retroviral capsid lattice stimulates the formation of a complementary lattice by TRIM5, with greatly increased TRIM5 E3 activity, and host cell signal transduction. Structural and biochemical studies on TRIM5 have opened a much needed window on how the innate immune system detects the distinct molecular features of HIV-1 and other retroviruses.

The tripartite motif (TRIM) family of proteins

TRIMs are multi-domain proteins defined by an N-terminal RING finger domain, one or two B-box domains, and a coiled-coil domain (Figure 1A). A large proportion of the TRIM proteins possess a C-terminal PRYSPRY domain that interacts with target proteins. Nearly 100 human genes encode TRIM proteins, and many of these are synthesized as multiple isoforms [1–3]. This enormous family of cellular proteins are involved in diverse cellular processes, including cell proliferation, differentiation, development, apoptosis, oncogenesis, and innate immunity. Of the many TRIM genes, several exhibit anti-retroviral activity, including TRIM11, 15, and 31 [4], TRIM1 [5,6], TRIM28 [7] and TRIM22 [8,9,9]. Among TRIM family members that inhibit HIV-1, TRIM5 is the best-studied.

TRIM5 and retrovirus restriction

TRIM5 is a cytoplasmic protein that blocks HIV-1 infection soon after the virus enters the target cell cytoplasm. It was discovered to be an HIV-1 restriction factor in functional, expression screens of cDNA libraries from macaque and owl monkey cells [10,11]. Cells

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from these species were targeted for study because they had particularly strong, well-characterized blocks to HIV-1 infection [12,13].

Once TRIM5 was cloned, it was found that, as compared with other species, the macaque and owl monkey TRIM5 orthologues associated relatively strongly with the HIV-1 virion core [14]. Interspecies variation in strength of TRIM5 binding to the capsid protein lattice of the virion core correlated with the ability of the TRIM5 orthologue from any given host species to block a given retrovirus. Lab strains of HIV-1 are weakly recognized by the human TRIM5 orthologue, which inhibits these viruses only 2-fold in single-cycle assays [10,15]. Compared to lab strains, though, some primary isolates are 10-fold more sensitive to restriction by human TRIM5 [16].

The clinical significance of TRIM5 for HIV-1 infection and disease progression in people is supported by several observations. TRIM5 polymorphisms and differences in expression influence rates of HIV-1 acquisition or disease progression [17–20]. HIV-1 variants that are highly sensitive to restriction by human TRIM5 appear to have been selected *in vivo* by pressure to escape from potent CTL targeting overlapping capsid determinants [21]. Additionally, a growing body of evidence indicates that TRIM5 in non-human primates plays an important role in limiting transmission of SIVs, or in controlling the outcome of infection with these viruses [22–26].

Capsid recognition by the PRYSPRY domain

Major determinants for capsid recognition are found in the C-terminus of TRIM5 (Figure 1A). In most species, TRIM5-mediated antiviral activity is associated with the *alpha* isoform. The C-terminus of this protein is a PRYSPRY (or B30.2) domain. PRYSPRY domains are found in over 500 different proteins and structures of PRYSPRY domains from the proteins sRFLPL1, TRIM21, GUSTAVUS, and PYRIN have been determined [27–30]. The common structure is a seven-stranded and a six-stranded antiparallel β -sheet, arranged in a β sandwich (Figure 1B). The loops that connect the β -strands form a surface that has been proposed to be the target specificity determinant.

The highly polymorphic PRYSPRY domain of the TRIM5 α isoform is a capsid-specificity determinant. This was demonstrated experimentally by testing the specificity of retrovirus restriction after swapping PRYSPRY domains among orthologues, as well as with phylogenetic comparisons [31,32]. In those cells that have been examined, 50% of TRIM5 transcripts encode alternative isoforms that lack a PRYSPRY domain, lack restriction activity, and act *in trans* to block the restriction activity of the alpha isoform [33]; in order of decreasing abundance, the mRNAs encoding these isoforms are called *iota*, *gamma*, *kappa*, or *delta*.

Direct, capsid-binding studies with TRIM5 α have been elusive because TRIM5 α recognizes the retroviral capsid protein lattice, not free capsid protein [14,34]. Efforts to detect CA binding to TRIM5 α have resorted to co-sedimentation with detergent-stripped virion cores mixed together *in vitro* [34], virion cores pelleted from the cytoplasm of infected cells [14], or tube assemblies generated *in vitro* from recombinant capsid protein [35]. Among other efforts to pinpoint sites of protein-protein interaction between TRIM5 and CA some investigators have attempted to stabilize minimal CA assemblies via oxidation of cysteine residues substituted at critical capsid interfaces [36,37]. These methods were critical to solving the atomic structure of the capsid hexamer and pentamer subunits that form the virion core [38]. Biochemical studies with these cross-linked components suggest that TRIM5 interacts with the trimer interface in the capsid lattice, not with the hexameric interface [35,39].

When visualized by electron microscopy after negative staining, recombinant rhesus TRIM5 α forms two dimensional crystals arranged in a hexagonal lattice [40]. TRIM5 mutants that block homo-dimerization or higher-order assembly, either of which disrupt restriction activity, prevented lattice formation. The efficiency of lattice formation was greatly increased when soluble TRIM5 protein was added to a planar lattice of HIV-1 capsid protein on an EM grid (Figure 1C). These fascinating observations suggest that complementary lattice formation plays an important role in TRIM5 restriction activity. Buttressing these biochemical observations, live-microscopy experiments detected accumulation of TRIM5 on virion cores as they arrive in the cytoplasm of a newly-infected cell [41].

Capsid recognition by cyclophilin A

All species of New World owl monkeys have a cyclophilin A domain at the C-terminus that was put there by LINE1-mediated retrotransposition of a cyclophilin A cDNA into the TRIM5 locus [11]. A similar TRIM5-cyclophilin A fusion gene in some species of Asian macaques was created by an independent retrotransposition event [42–45]. Prior to the discovery of TRIM5, free cyclophilin A protein had been demonstrated to be an HIV-1 capsid binding protein [46,47]. Consistent with this, disruption of the cyclophilin domain destroyed HIV-1 restriction by owl monkey TRIM5Cyp [11] and a potent, HIV-1-specific inhibitor was engineered by fusing human TRIM5 to human cyclophilin A [48].

Cyclophilin A orthologues from *Saccharomyces cerevisiae* to *Homo sapiens* are functionally interchangeable [49] and orthologues from all species that have been examined retain the ability to bind HIV-1 capsid, but not HIV-2 capsid [11,50]. The owl monkey TRIM5-cyclophilin A fusion protein restricts HIV-1, but not HIV-2 [11,51]. It was therefore quite surprising when the TRIM5-cyclophilin A fusion protein from macaques was found to neither bind HIV-1 capsid nor restrict HIV-1 replication [43,44]; instead, it restricts HIV-2. The overall topology of the HIV-1 and HIV-2 capsids is the same, but the orientation of the critical residues linking helix 4 and 5 is very different [52]. X-ray crystallography, in combination with functional analysis of mutants, showed that a single amino acid change in the cyclophilin domain, D66N, confers these specificity differences [52]. Loss of constraining interactions from the aspartate residue frees a loop of 10 residues, such that its orientation is shifted by 16Å (Figure 2). This demonstrates that cyclophilin has an extraordinary capacity to alter its mode of substrate binding and perhaps explains the utility and widespread presence of this modular unit in many different cellular proteins.

The RING domain

The RING domain of TRIM5 functions as an E3 ubiquitin ligase [39,53–55]. The NMR solution structure was determined for a fragment of TRIM5 that comprises amino acid residues 1 to 78 (Figure 3), encompassing the RING domain [56]. It reveals a compact $\beta\beta\alpha$ RING-fold core, with residues 10 to 61 interacting with residues 62 to 70. Two Zn²⁺ ions are coordinated by zinc-fingers, one of which is coordinated by 4 cysteines and the other by a histidine and 3 cysteines. Hydrophobic residues are mostly packed in the core of the structure. However, some of the hydrophobic residues are solvent exposed. Two hydrophobic surface patches have been defined, the RING-RING interaction region and the E2 binding region of TRIM5. Mutations have been identified in the latter that disrupt retrovirus restriction activity and TRIM5 auto-ubiquitination in the presence of E2 UBCH5B, without consequence to other biochemical functions essential for restriction, such as TRIM5 association with capsid or with itself [56]. These structure-function studies indicate that RING E3 ubiquitin ligase activity is necessary for retrovirus restriction activity.

The B-box domain

All TRIM proteins have either one or two B box domains [1,2]. TRIM5 has only one. Generally, B-box domains are involved in protein-protein interactions. In TRIM proteins, these domains cooperate with the coiled-coil domain, to form higher-order oligomers and cytoplasmic bodies. Mutations in the TRIM5 α B-box-2 domain prevent the higher-order multimerization required for efficient capsid binding and restriction activity [57,58]. Amino acid residues in the linker domain between the coiled-coil and PRYSPRY domains also contribute to these activities [59].

The NMR solution structure of a fragment of the TRIM5 protein (residues 86–131) that comprises the B-box 2 domain showed a $\beta\beta\alpha$ RING-like fold with two Zn^{2+} ions in a CHCDC2H2 coordination mode [58] (Figure 4). An additional short β -strand precedes the helix. TRIM B-box domains commonly have two hydrophobic surface patches. Cluster 1 is located on the outer surface of the helical part and the first β -strand (TRIM5 α residues L103, L104, W115, and L116) and is surrounded by charged residues. Cluster 2 is adjacent to cluster 1, and includes residues L102, V112, F105, F129. Mutations within cluster 1, in particular of Arg121, indicate that this surface region is responsible for most of the biological effects attributed to the B-box 2 domain in TRIM5.

The coiled-coil domain

Sequence comparisons revealed a likely coiled-coil domain in the region between the B-box 2 domain and the PRYSPRY domain of TRIM5 (Figure 1A). From the standpoint of structure, this domain is perhaps the least well explored and the exact boundaries are not well defined due to lack of sequence homology with other coiled-coil proteins. The coiled-coil domain in TRIM proteins is responsible for dimerization [35,55]. A structure of this domain and its interaction with the other domains of TRIM proteins would be extremely useful for understanding the function of TRIM5, especially considering that the coiled-coil is absolutely essential for restriction activity.

Structure of full-length TRIM5

As discussed above, the domain composition and a series of biochemical experiments suggest that TRIM5 exists as a dimer (via the coiled-coil domain) with a tendency to form higher oligomeric forms (via the B box 2 domain and the linker 2 region). Despite significant progress on the structure of the isolated TRIM5 domains, full-length TRIM5 protein has proved difficult to express and purify in a soluble form. Therefore, the spatial arrangement of the domains within the context of the full-length protein is unknown. Recombinant rhesus TRIM5 α in which the N-terminal RING domain is replaced by the TRIM21 RING domain (TRIM5-21R) is better behaved *in vitro* than the wild-type protein [55]. Since TRIM5-21R retains retroviral restriction activity like that of rhesus TRIM5 α [53] it has been used as a clever surrogate for full-length TRIM5. Recently, full-length, dimeric TRIM5 was expressed and purified in sufficient quantities for biochemical analysis, *in vitro* assessment of its enzymatic activity, and protein-protein interaction studies with capsid and other cellular proteins [39]. To date, though, there is no three-dimensional structural information for the full-length protein.

Mechanism of retrovirus restriction

The exact mechanism of retrovirus restriction by TRIM5 is not known. Pairing of select TRIM5 orthologues with retroviruses for which capsid avidity is highest - for example, rhesus macaque TRIM5 α with HIV-1, or human TRIM5 α with N-tropic MLV - results in disassembly of the incoming capsid before completion of reverse transcription [32]. This

block before reverse transcription requires TRIM5 E3 ubiquitin ligase activity [39,56], and possibly recruitment of proteasomes via direct contact of TRIM5 with the proteasome component PSMC2 [60], or via the adaptor protein p62/sequestosome 1 (SQTM-1) [61]. TRIM5 is autoubiquitinated and degraded in response to restriction sensitive virus [53,56,62]. Despite these observations, TRIM5-mediated ubiquitination of HIV-1 capsid has not been detected. Understanding the restriction mechanism is confounded further by the fact that, if the block to reverse transcription is bypassed, replication steps after reverse transcription are also blocked by TRIM5 [63,64]. Particularly intriguing is the report that leptomycin traps the human and rhesus orthologues of TRIM5 α in the nucleus [65], indicating that these proteins shuttle from the nucleus to the cytoplasm in a CRM1-dependent manner.

Innate immune signaling and TRIM5-mediated restriction

TRIM5 restriction activity is cell-type specific [66,67], and the isolation of restriction-defective, TRIM5-positive cell lines suggested that TRIM5 restriction requires specific host co-factors [68]. Additional clues about the TRIM5 restriction mechanism came from the finding that retroviruses are rescued from restriction by drugs that block signal transduction [67,69,70]. Indeed, TRIM5 promotes AP-1 and NF- κ B signaling [39,71] (Figure 4), and knockdown of TRIM5 in dendritic cells prevents innate immune signaling downstream of LPS and other pathogen-associated molecular patterns [39].

Dissection of the mechanism by which TRIM5 promotes signaling showed that it acts with the E2 enzymes UBC13/UEV1A (also known as UBE2N–UBE2V1) to catalyze the synthesis of unattached, K63-linked ubiquitin chains that activate the TAK1 kinase complex and, consequently, the transcription of inflammatory cytokines [39] (Figure 5). It was then demonstrated that TAK1, UBC13 and UEV1A contribute to the restriction mechanism. Reciprocally, interaction with HIV-1 capsid lattice tubes, but not with free capsid protein or crosslinked capsid hexamers, greatly enhances the UBC13–UEV1A-dependent E3 activity of TRIM5 and the induction of inflammatory cytokines [39]. These findings demonstrate that TRIM5 is a pattern recognition receptor (PRR) specific for the protein lattice of the retrovirus capsid.

It is becoming clear that many TRIM family members regulate innate immune signaling, and that many of these TRIMs will be important HIV-1 regulators [72]. Recently it was shown that, when the TREX1 nuclease is disrupted, the viral cDNA of HIV-1 and other retroviruses is detected by an innate immune receptor in the host cell cytoplasm, resulting in strong induction of type 1 interferon [73,74]. The identity of the DNA receptor has not been determined, but the signaling pathway downstream of this DNA receptor requires K63-linked ubiquitination of STING by TRIM56 [74,75] (Figure 5).

HIV-1 genomic RNA is highly structured [76,77], and bears an unusual trimethylguanosine cap [78]. One can speculate that, under conditions analogous to those where TREX1 is disrupted, HIV-1 RNA would be detected by an innate immune sensor. Some studies suggest that RIG-I senses HIV-1 RNA and that the HIV-1 protease blocks innate immune signaling in response to HIV-1 RNA by binding and sequestering RIG-I [79,80]. Interestingly, RIG-I signaling is also activated by K63-linked ubiquitin, and this requires TRIM25 [81,82] (Figure 5).

Concluding remarks

The complementarity of the TRIM5 lattice for the capsid suggests that TRIM5 recognition involves low-affinity binding sites distributed across the capsid hexameric lattice [40]. This would explain how given TRIM5 orthologues restrict retroviruses bearing capsids of heterogeneous amino acid sequence and geometry. A full understanding of the restriction

mechanism will necessitate solution of the full TRIM5 structure in complex with the capsid lattice. Restriction activity may involve premature disassembly of the capsid lattice with blockade of reverse transcription [10] and targeting of the TRIM5-capsid complex to the proteasome [60,62]. Alternatively, the TRIM5 lattice might stabilize the capsid and prevent uncoating or reverse transcription, or block capsid interaction with host machinery essential for subsequent steps in infection such as nuclear entry [63,64]. With either scenario, free K63 Ub chains generated by UBC13, UEV1A and capsid-hexamerized TRIM5 contribute to restriction activity by activating TAK1 [39]. Identification of TAK1 substrates, whether viral proteins or host effector proteins, will inform future attempts to clarify the mechanism of restriction. It will be interesting to determine if other HIV-1 restriction factors such as APOBEC3G and TETHERIN also signal in response to HIV-1 infection, or if restriction activity by these factors also depends upon any of the hundreds of other TRIM family members.

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Highlights

- TRIM5, with UBC13/UEV1A, catalyzes the synthesis of free K63-linked ubiquitin chains.
- TRIM5 activates the TAK1 (MAP3K7) complex
- TAK1, UBC13, and UEV1A are required for TRIM5-mediated restriction
- TRIM5 spontaneously forms a two-dimensional paracrystalline hexagonal lattice
- HIV-1 capsid lattice promotes TRIM5 assembly into a lattice
- Capsid lattice stimulates TRIM5 E3 activity and signal transduction

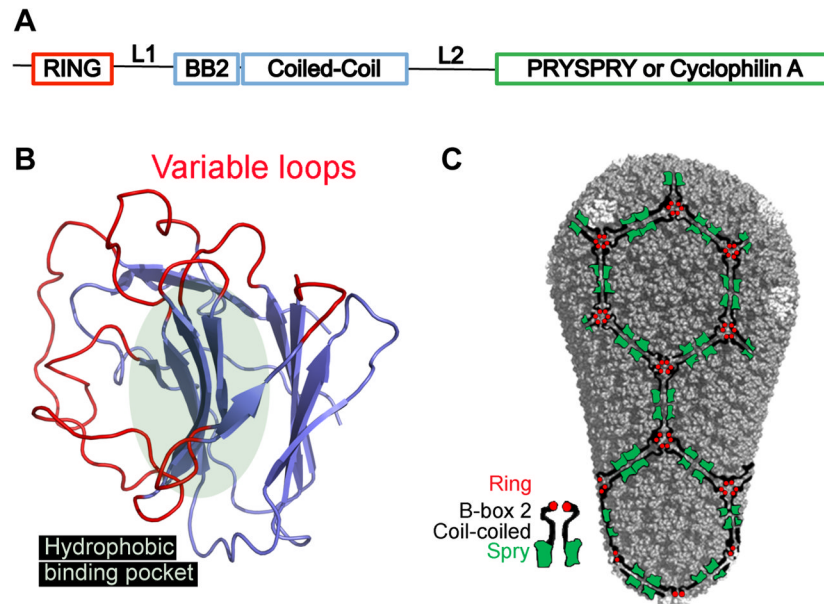


Figure 1.

Figure 1A: Schematic representation of the domains within TRIM5, with relative positions of the domains along the linear sequence indicated. RING, really interesting new gene; L1, linker 1; BB2, B box 2; L2, linker 2.

Figure 1B. Ribbon diagram of a model of the human TRIM5 α PRYSPRY (B30.2) domain. Variable loops extending from the β -sandwich are marked in red, the putative hydrophobic binding pocket is indicated by the light green oval.

Fig. 1C. Model of the HIV-1 capsid fullerene cone with hexagonal TRIM5 α lattice superimposed, illustrating a putative recognition mode of capsid by TRIM5 α , as in reference [40]. The color code for the TRIM5 α domains is indicated in the schematic model of the TRIM5 α dimer at the bottom left of the panel.

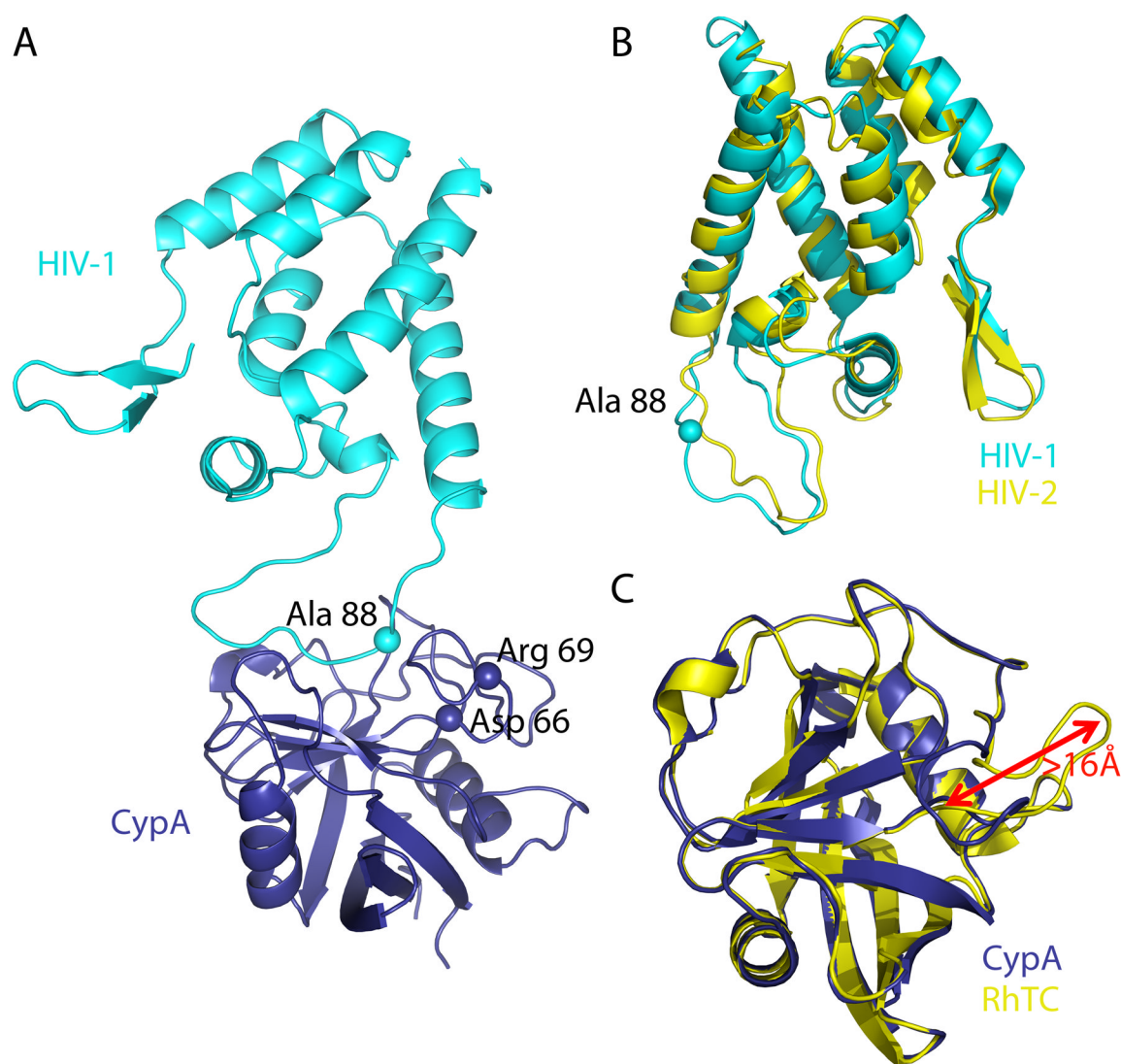


Figure 2.

Figure 2A: Cartoon representation of HIV-1-CypA complex (PDB 1AK4, Chains A and D) in which HIV-1 is in cyan and CypA is in dark blue. The proximity of HIV-1 CA residue Ala 88 to the CypA residues Asp 66 and Arg 69 (spheres) is shown.

Figure 2B: Cartoon representation of the N-terminal capsid domains of HIV-1 (PWB 1GWP, first NMR model) in cyan and of HIV-2 (PDB 2WLV, chain A) in yellow after SSM superposition. The cyan sphere indicates the position of Ca Ala 88 in HIV-1, which is deleted in HIV-2.

Figure 2C: Cartoon representation of CypA (PDB 2CPL) in dark blue SSM superimposed on RhTC (PDB 2WLW) in yellow. Loop 64–74, which contains amino acid replacements D66N and R69H has undergone a rearrangement of $>16\text{\AA}$.

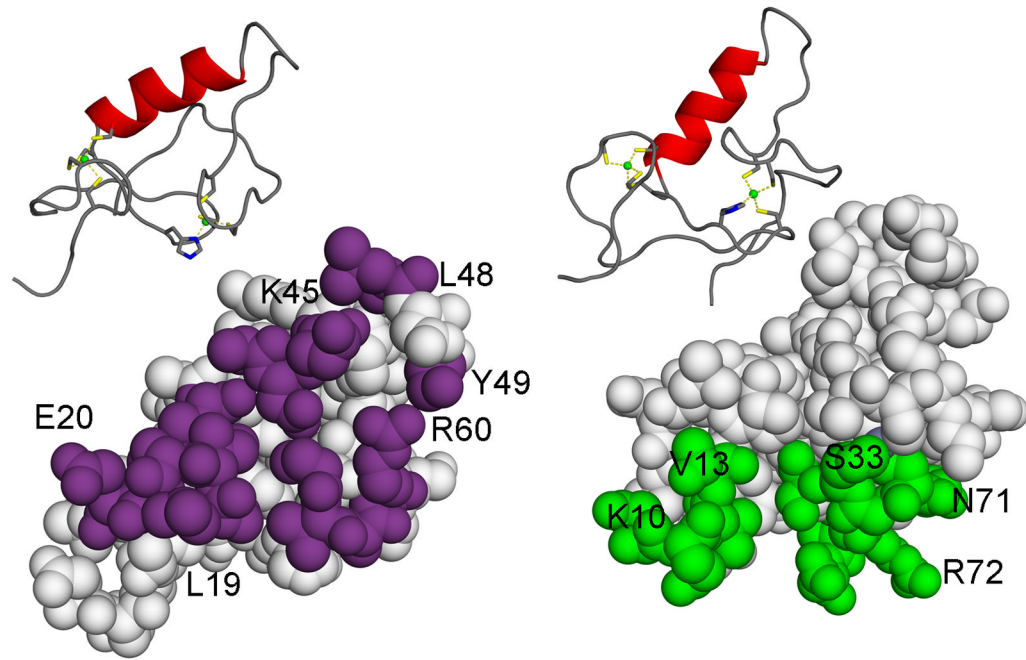


Figure 3.
 NMR solution structure of human TRIM5 α , amino acid residues 1 to 78, encompassing the RING domain [56].
Top: ribbon diagrams with Zn²⁺ ions indicated as green balls. Coordinating residues are highlighted.
Bottom: surface representations.
Left: the RING-RING interaction region is indicated in magenta.
Right: the E2 binding region is indicated in green.

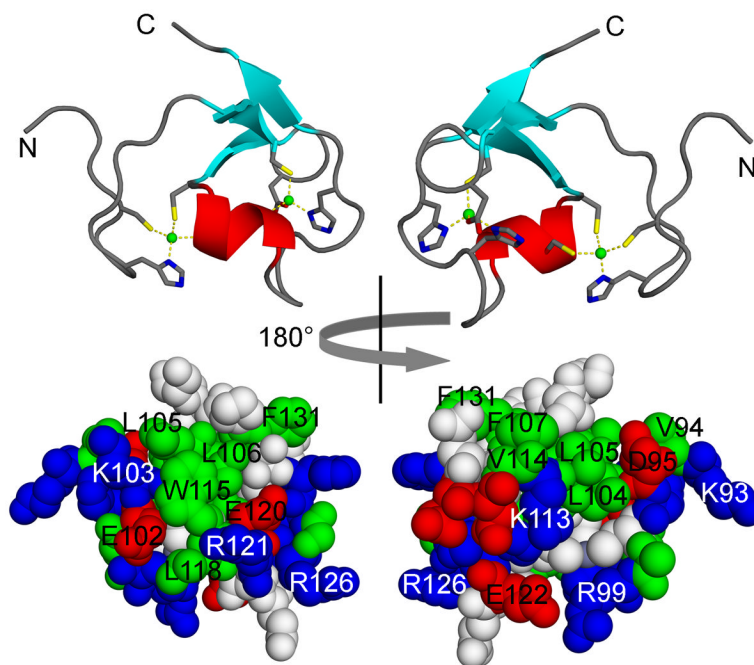


Figure 4. NMR solution structure of human TRIM5 α amino acid residues 86 to 131, encompassing the B-box 2 domain [58].
Top: ribbon diagrams with Zn²⁺ ions indicated as green balls, in a CHCDC2H2 coordination mode. Coordinating residues are highlighted.
Bottom: surface representations.
Left: hydrophobic surface cluster 1.
Right: hydrophobic surface cluster 2.

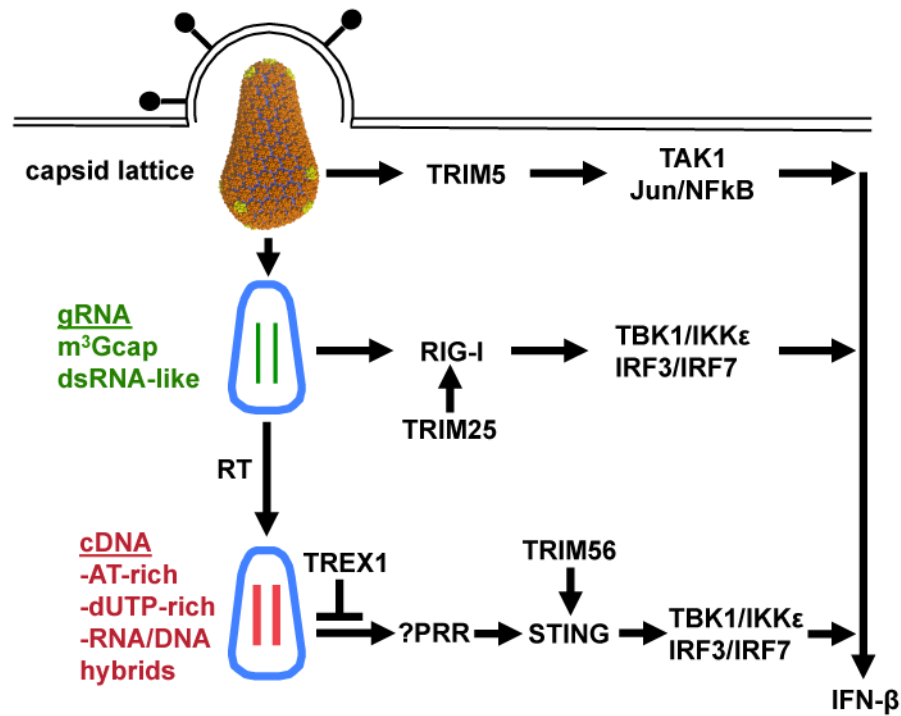


Figure 5.

Schematic diagram showing involvement in the innate immune response to HIV-1 of some of the many TRIM proteins. The role of TRIM5 has been confirmed, and, based on the evidence, the roles of TRIM25 and TRIM56 are likely.

TRIM5 recognition of the capsid lattice stimulates its E3 ubiquitin ligase activity [39]. This activates TAK1 and downstream NF-κB and AP-1 factors. Such signalling is required for, but not sufficient to activate, interferon-beta transcription.

TRIM25 is required for signaling by the RIG-I pattern recognition receptor [81,82]. RIG-I responds to the highly, structured HIV-1 genomic RNA [79,80].

TRIM56 is required for STING-dependent signalling from the cytoplasmic pattern recognition receptor for DNA [75], that is activated when HIV-1 infects TREX1-deficient cells [74].