TRIM5alpha

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Contents

Abstract TRIM5α protein blocks retroviral replication at early postentry stage reducing the accumulation of reverse transcriptase products. TRIM5α proteins of Old World primates restrict HIV-1 infection whereas TRIM5α proteins of most New World monkeys restrict SIV_{mac} infection. TRIM5α protein has a RING domain, B-box 2 domain, coiled-coil domain, and PRYSPRY domain. The PRYSPRY domain of TRIM5α determines viral specificity and restriction potency by mediating recognition of the retroviral capsid. The coiled-coil domain is essential for TRIM5α oligomerization, which contributes to binding avidity for the viral capsid. The RING domain and B-box 2 domain are required for efficient restriction activity of TRIM5α protein but the mechanisms remain to be defined.

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1 Introduction

Following entry into the host cells, retroviruses must execute a series of processes including the uncoating of the viral core, reverse transcription, nuclear import, and integration of the viral DNA into the host genome before they establish a successful infection (Arts and Wainberg 1996; Whitcomb and Hughes 1992). The prevalence of retroviral DNAs in the genomes of all eukaryotes suggests that they have been present throughout the course of evolution, and about 8% of the human genome is made of the relics of past infections in the form of extinct endogenous retroviruses (Bannert and Kurth 2004). The integration of viral DNA and expression of viral proteins are potentially mutagenic or pathogenic. Therefore, the ability of an organism to limit or restrict retrovirus replication should have a selective edge over virus-susceptible counterparts. There are multiple genetic barriers to HIV-1 replication in most nonhuman primates hampering our efforts in developing a robust animal model for HIV-1 infection and pathogenesis. A major cellular factor responsible for blocking HIV-1 infection in Old World monkeys at early postentry step was identified as TRIM5α by Sodroski and colleagues in 2004 (Stremlau et al. 2004). Our efforts in understanding how TRIM5α interferes with the replication cycle of HIV-1 will hopefully allows us to manipulate this system to induce antiviral states in the near future. What follows is a brief review of the postentry restrictions of retroviruses, followed by highlights of these recent new developments on the antiretroviral activity of TRIM5α.

2 Postentry Restrictions

2.1 Fv1

Evidence of a specific, postentry restriction of retroviruses was first provided by studies of murine leukemia virus (MLV) replication in mouse cells of different genetic backgrounds regarding the *Fv1* gene, which was first identified as a locus that controlled susceptibility to Friend leukemia virus disease (Lilly 1970; Pincus Hartley and Rowe 1971). The virus resistance induced by *Fv1* was genetically dominant over susceptibility and was evident in cells cultured in vitro. Among a variety of *Fv1* alleles, two alleles of *Fv1* were shown to provide resistance to infection by particular MLV types. The $Fv1^b$ allele, present in Balb/c mice, blocks infection of N-MLV, whereas the $Fv1^n$ allele, present in NIH/swiss mice, blocks infection of B-MLV. This block occurs after reverse transcription but prior to integration, and targets the MLV capsid (DesGroseillers and Jolicoeur 1983; Jolicoeur and Rassart 1980; Ou et al. 1983; Pryciak and Varmus 1992; Sveda and Soeiro 1976). Indeed, a single amino acid residue at position 110 in the viral capsid can determine the susceptibility of the virus to the blocking effects of different *Fv1*

alleles (Kozak and Chakraborti 1996). Fv1 activity is saturated or titrated at high multiplicity of infection.

Fv1 gene arose from the germ-line integration of an endogenous retrovirus and encodes a Gag-like product (Best et al. 1996). An intact sequence corresponding to the major homology region (MHR), which is conserved in all retroviral capsids and contributes to capsid–capsid interactions, is important for Fv1 function (Bishop et al. 2001). However, the mechanism of the Fv1-mediated block is not understood. It is possible that Fv1 interferes with the trafficking of preintegration complex (PIC) or inhibits the integration of PIC into the host chromosome.

2.2 *Ref1 and Lv1*

In addition to governing the ability of retroviruses to infect particular mouse strains, early postentry restriction can also determine tropism at the species level. N-MLV, for example, inefficiently infects human cells and certain cell lines from African green monkeys (Besnier et al. 2003; Towers et al. 2000). The cellular factor restricting N-MLV in human cells has been referred to as Ref1 (restriction factor 1). As for Fv1 mediated restriction, the major determinant for virus susceptibility to Ref1 was the amino acid 110 of the capsid protein, and Ref1 blocked N-MLV in a saturable manner. However, Ref1 blocked infection at a slightly earlier stage than Fv1, before reverse transcription.

HIV-1 encounters a postentry block in Old World monkeys, whereas simian immunodeficiency virus (SIV_{mac}) is blocked in most New World monkey cells (Himathongkham and Luciw 1996; Hofmann et al. 1999; Shibata et al. 1995). The cellular factor dictating the susceptibility of primate cells to the lentiviruses was referred to as Lv1 (lentiviral susceptibility factor 1) (Cowan et al. 2002). Lv1 restricted HIV-1 in a saturable manner (Hofmann et al. 1999; Towers et al. 2000). Like the restriction mediated by Ref1, the Lv1 restriction occurred early after entry, before reverse transcription, and the resistance was dominant over sensitivity (Cowan et al. 2002; Munk et al. 2002). As for Fv1 and Ref1, the determinant for virus susceptibility was the viral capsid protein (Cowan et al. 2002; Dorfman and Gottlinger 1996; Hatziioannou et al. 2003; Owens et al. 2003) and the block could be abrogated with wild-type HIV-1 (Kootstra et al. 2003) or with replication-defective particles lacking reverse transcriptase activity (Besnier et al. 2003; Cowan et al. 2002).

These species-specific, postentry restrictions mediated by Ref1 and Lv1 share common features: (1) the block occurs prior to reverse transcription (Cowan et al. 2002; Himathongkham and Luciw 1996; Munk et al. 2002; Shibata et al. 1995); (2) the viral determinant of the susceptibility to restriction is the capsid protein (Cowan et al. 2002; Kootstra et al. 2003; Owens et al. 2003; Towers et al. 2000); and (3) the host cell restricting factor can be competed by virus-like particles containing proteolytically-processed capsid proteins of the restricted viruses (Besnier Takeuchi and Towers 2002; Cowan et al. 2002; Hatziioannou et al. 2003; Owens et al. 2004).

3 TRIM5α

3.1 Identification of TRIM5α Restriction

While the search for the genes encoding Ref1 and Lv1 continued, the similarities in the nature and timing of the block imposed by the two loci raised the possibility that Ref1 and Lv1 might be human and monkey versions of the same gene. This possibility was further supported by a cross-abrogation experiment in African green monkey (AGM) cells which show a broad range of restriction against retroviruses including HIV-1, HIV-2, EIAV, and N-MLV. For example, the restriction of HIV-1 in AGM cells could be abrogated by EIAV, and the restriction of N-MLV could be abrogated by the lentiviruses (Besnier et al. 2002; Hatziioannou et al. 2003; Stoye 2002; Towers et al. 2000).

A major breakthrough in the field was accomplished with the identification of the gene responsible for Lv1 activity (Stremlau et al. 2004). TRIM5α was identified during a screen for cDNA clones derived from HIV-resistant, rhesus macaque lung fibroblasts that would protect human HeLa cells from infection by single-cycle GFP-expressing HIV-1 vector pseudotyped with VSV-G envelope when introduced into HIV-susceptible human HeLa cells. The promiscuous envelope protein VSV-G allows entry into most mammalian cell types and thus bypasses blocks related to cell-surface binding, fusion, and entry. This particular screen, therefore, specifically revealed the presence of barriers to the first half of the retroviral life cycle, including reverse transcription, integration, and expression. The expression of the rhesus cDNA was sufficient to restrict incoming HIV-1, whereas the human cDNA was not. Rhesus TRIM5α activity was specific for the HIV-1 capsid, as expected for Lv1 activity. In the cells expressing rhesus TRIM5α, the accumulation of reverse transcripts was significantly reduced indicating that TRIM5α blocks HIV-1 replication before or during early reverse transcription (Fig. 1). Furthermore, siRNA-mediated knockdown of endogenous TRIM5α expression in rhesus cells abrogated the early postentry restriction to HIV-1 infection indicating that TRIM5α was required for Lv1 activity. Work performed by several laboratories soon confirmed that Ref1 and Lv1 were indeed the human and monkey orthologues of TRIM5α (Hatziioannou et al. 2004b; Keckesova Ylinen and Towers 2004; Perron et al. 2004; Song et al. 2005c; Yap et al. 2004).

3.2 TRIM5α

TRIM5α is a member of the large family of tripartite motif proteins (TRIM) (Reymond et al. 2001). TRIM proteins contain RING, B-box 2, and coiled-coil domains and thus have been referred to as RBCC proteins (Reymond et al. 2001) (Fig. 2). Human TRIM5 gene is located in chromosome 11p15 in a cluster with other

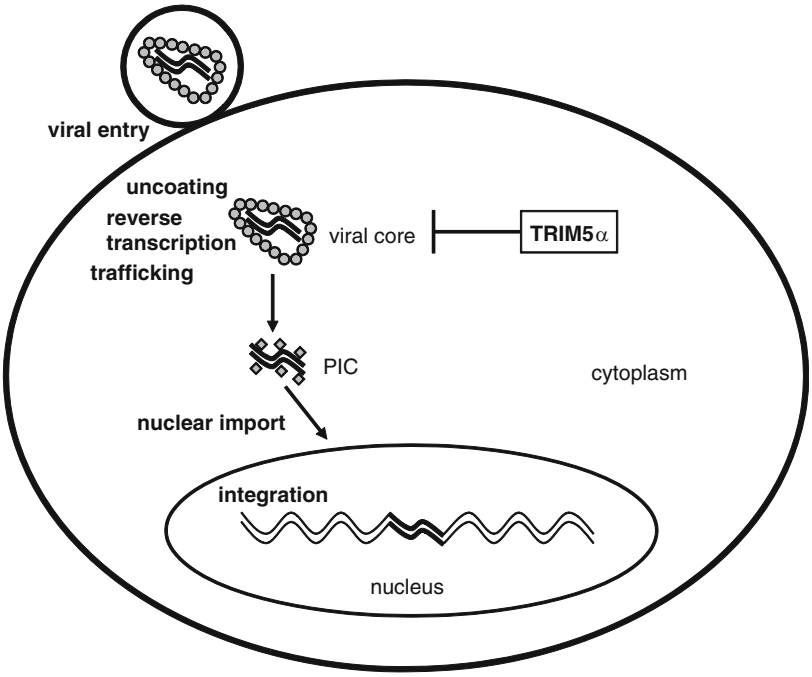

Fig. 1 Postentry restriction of retroviral infection by TRIM5α. The early steps of retrovirus replication cycle and the position of block generated by TRIM5α are shown. *PIC* Preintegration complex

TRIM genes including TRIM3, TRIM6, TRIM21, TRIM22, TRIM34, and TRIM68 (Fig. 2). Among these TRIM genes, TRIM5, TRIM6, TRIM22, and TRIM34 are located at adjacent loci. TRIM5α displays higher identities to adjacent TRIM proteins in RING and B-box domains, but lower identities in coiled-coil and PRYSPRY domains. Consistent with this observation, the carboxy-terminal PRYSPRY domain has recently been shown as a variable region that determines the species specificity of retroviral restriction in primates.

Differential splicing of the TRIM5 primary transcript gives rise to the expression of several isoforms of the protein product (Reymond et al. 2001). The TRIM5α is the largest product (493 amino acid residues in humans) and contains the PRYSPRY domain. The other TRIM5 isoforms lack an intact PRYSPRY domain and are incapable of restricting HIV-1. Two TRIM5 isoforms, TRIM5δ and TRIM5α, are reported to have ubiquitin ligase activity typical of RING-containing proteins (Xu et al. 2003; Yamauchi et al. 2008).

TRIM proteins often self-associate and form nuclear or cytoplasmic bodies of undefined function (Diaz-Griffero et al. 2006; Reymond et al. 2001; Song et al. 2005a). Although TRIM proteins have been implicated in transcriptional regulation,

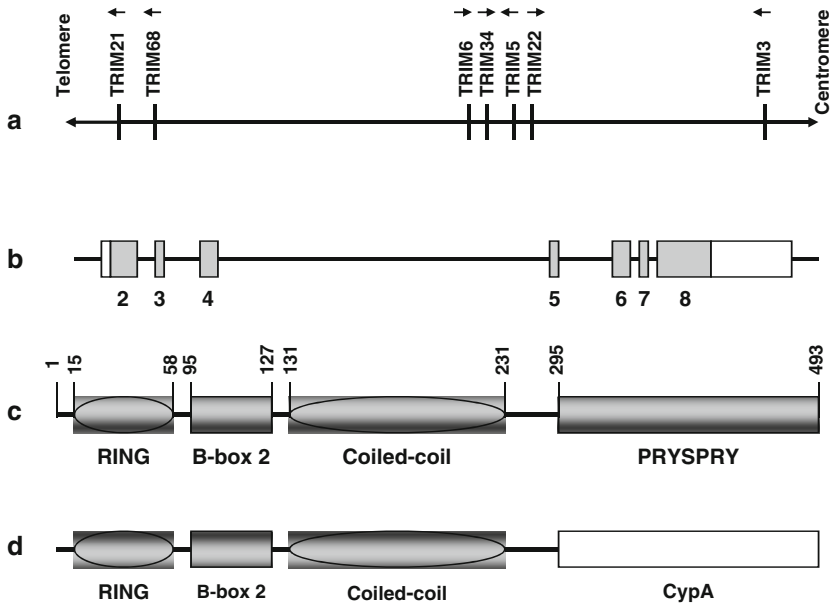

Fig. 2 Chromosomal localization and domain structure of TRIM5α. (**a**) TRIM genes in the segment of 11p15, which is present in the distal region of human chromosome 11. (**b**) Schematic representation of TRIM5 gene. The coding sequences for the alpha isoform is spread across 7 exons, beginning with the RING domain in exon 2 and ending with the PRYSPRY domain in exon 8. (**c**) Schematic representation of the domain structure of human TRIM5α protein. Other isoforms such as TRIM5γ or TRIM5δ do not have the PRYSPRY domain and they do not restrict retroviruses. (**d**) Schematic representation of the domain structure of TRIMCyp fusion protein expressed in owl monkey cells. TRIMCyp fusion consists of the RBCC domain of TRIM5 fused to cyclophilin A due to the insertion of a CypA sequence between exons 7 and 8

cell division, antiviral activity, determination of cell polarity, and differentiation, the precise functions of most TRIM proteins remain to be determined (Meroni and Diez-Roux 2005; Nisole et al. 2005; Towers 2005). TRIM proteins arose with the metazoans and have expanded in number during vertebrate evolution (Reymond et al. 2001). To date, more than 70 TRIM proteins have been identified in the human genome; homologues exist in other species as well. Dysregulation and mutations of some TRIM family members have been linked to a variety of pathological conditions, including genetic diseases and oncogenesis. Many TRIM proteins, including TRIM19, TRIM21, TRIM22, TRIM34, and TRIM5α itself, can be upregulated by interferon, supporting their potential role as effectors in the antiviral cellular response (Asaoka et al. 2005; Chelbi-Alix et al. 1995; Gongora et al. 2000; Orimo et al. 2000; Tissot and Mechti 1995). Indeed, several primate TRIM proteins, including TRIM1, TRIM5α, TRIM19, TRIM22, TRIM32, and TRIM34 have been shown to have antiviral activity against different viruses (Nisole et al. 2005).

3.3 *TRIMCyp*

Cyclophilin A (CypA) is a highly conserved peptidyl prolyl isomerase that binds to HIV-1 capsid (CA) (Franke et al. 1994; Luban et al. 1993; Thali et al. 1994). HIV-1 directly interacts with the CypA activie site by virtue of residues in a loop between the fourth and fifth alpha helices of CA (4–5 loop) (Bukovsky et al. 1997; Gamble et al. 1996). A proline residue P90 in HIV-1 CA is required for CypA binding (Franke et al. 1994). Blocking the CypA-CA interaction by either mutation of the critical proline itself or nearby residues in CA, by mutation of the cyclophilin gene, or by addition of the drug cyclpsporin inhibited virus replication in human cells (Braaten et al. 1996; Braaten and Luban 2001; Dorfman and Gottlinger 1996; Franke et al. 1994; Thali et al. 1994). The affected step was early after entry, before reverse transcription, at the same time as the Ref1 and Lv1 blocks. Analysis of the retroviral replication cycle, using RNA interference to disrupt CypA in the virion producer cell or in the target cell, indicated that target cell CypA alone promotes HIV-1 infectivity (Kootstra et al. 2003; Towers et al. 2003).

Owl monkey cells restricted HIV-1 whereas most other New World monkey cells blocked SIV_{mac} infection (Hofmann et al. 1999). In contrast to the positive effect of CypA-CA interaction on HIV-1 replication in human cells, Towers et al. showed that inhibiting CypA in owl monkey cells rescued HIV-1 restriction (Towers et al. 2003). This was later explained by the identification of TRIM5-cyclophilin A fusion protein (TRIMCyp) (Nisole et al. 2004; Sayah et al. 2004), which arose by retrotransposition of a complete CypA cDNA into TRIM5 intron 7 (Fig. 2). These findings raised a possibility that the CypA domain in the owl monkey TRIMCyp and the PRYSPRY domain in the rhesus TRIM5α could provide a binding domain to target the incoming HIV-1 core and the N-terminal domain(s) of TRIM5α and TRIMCyp could serve an effector function.

It was also shown that inhibiting CypA in Old World monkey cells reduced HIV-1's sensitivity to TRIM5α (Berthoux et al. 2005; Keckesova et al. 2006; Kootstra et al. 2003; Stremlau et al. 2006b) and this was concluded to be due to CypA-mediated prolyl isomerization of CA residue P90 impacting on sensitivity to TRIM5α binding (Berthoux et al. 2005; Keckesova et al. 2006). It was also reported that TRIM5α does not have a role for CypA sensitivity of HIV-1 in human cells (Hatziioannou et al. 2005; Keckesova et al. 2006; Sokolskaja et al. 2006).

Initially, a TRIMCyp fusion gene was thought to exist only in the owl monkey, a New World monkey. However, recent studies reported that the TRIMCyp fusion gene is also found in Old World monkeys including rhesus macaque and pig-tailed macaque (Brennan et al. 2008; Liao et al. 2007; Newman et al. 2008; Virgen et al. 2008; Wilson et al. 2008). Rhesus TRIMCyp restricts infection of HIV-2 and FIV but not HIV-1 (Wilson et al. 2008) and pig-tailed TRIMCyp restricts FIV but not HIV-1 (Brennan et al. 2008; Virgen et al. 2008). TRIMCyp genes of Old World monkeys were proposed to be generated independently from that in owl monkeys as indicated by different position of CypA cDNA sequence, and these events constitute a remarkable example of convergent evolution.

4 Restriction Activity of TRIM5α

4.1 Interspecies Variation of TRIM5α and Retroviral Restriction

Sequence analysis revealed significant interspecies variability in the PRYSPRY domains of TRIM5α proteins of Old World and New World monkeys (Sawyer et al. 2005; Song et al. 2005b). These studies showed substitution patterns indicative of selection in the PRYSPRY domain and revealed lineage-specific expansion and sequential duplication in the PRYSPRY domain (Song et al. 2005b). For the sequences encoding the PRYSPRY domain, the Ka/Ks ratio was very high, indicative of selectively driven diversity. These results suggest that occasional, complex changes were incorporated into the TRIM5α PRYSPRY domain at discrete time points during the evolution of primates. Some of these time points correspond to periods during which primates were exposed to retroviral infections, based on the appearance of particular endogenous retroviruses in primate genomes.

Soon after the identification of TRIM5α as a restriction factor blocking HIV-1 replication at early postentry steps in rhesus monkey cells, several laboratories cloned the TRIM5α cDNAs from diverse primate species and tested their antiretroviral activities (Table 1) (Hatziioannou et al. 2004b; Newman et al. 2006; Ohkura et al. 2006; Perez-Caballero et al. 2005a; Sawyer et al. 2005; Song et al. 2005a, 2005c; Stremlau et al. 2004; Yap et al. 2004). The ability of TRIM5α proteins from different primate species to restrict infection by various retroviruses (Table 1) and the evidence for positive evolutionary selection of TRIM5 genes support the possibility that a major natural function of TRIM5α is its antiviral activity.

Recent studies identified TRIM5 genes with restriction activity against divergent retrovirus in cows (Si et al. 2006; Ylinen et al. 2006) and rabbits (Schaller et al. 2007) and revealed several TRIM5-like genes in rodents (Tareen et al. 2009), and

Table 1 Restriction of retroviruses by primate TRIM5alpha proteins

Group	Species	HIV-1	SIV_{mac}	N-MLV
Hominoids	Human	−/+	−	++
	Chimpanzee	−/+	−	++
	Gorilla	−/+	+	++
	Orangutan	−/+	−/+	++
Old World monkeys	Rhesus macaque	++	+	++
	Pigtailed macaque	++	−	++
	AGM(pyg)	++	−	++
	AGM(tan)	++	++	++
	Sooty mangabey	++	−	++
New World monkeys	Squirrel monkey	−	++	−
	Tamarin (red-chested)	+	++	+
	Tamarin (cotton top)	+	++	++
	Tamarin (emperor)	+	++	++
	Spider monkey	++	++	++

− No restriction, + weak restriction, ++ strong restriction, −/+ no restriction or weak restriction

phylogenetic analysis of these TRIM genes suggest that these factors have evolved from a common ancestor with antiretroviral properties and have undergone independent evolutionary expansions within species.

4.2 Role of TRIM5α Domains

The RING domain, a cycteine-rich zinc binding sequence, found at the N-terminus of TRIM5α is involved in specific protein–protein interactions and often associated with E3 ubiquitin ligase activity (Freemont 2000; Pickart 2001). Indeed, ubiquitination of TRIM5α (Diaz-Griffero et al. 2006; Yamauchi et al. 2008) and TRIM5δ (Xu et al. 2003) has been demonstrated. It was shown that TRIM5 is able to ubiquitinate itself in a RING domain-dependent manner (Xu et al. 2003; Yamauchi et al. 2008). Deletion of the RING domain as well as point mutations affecting residues known to be critical for ubiquitin ligase activity (C15 and C18) significantly reduced the HIV-1 restriction activity of rhesus TRIM5α (Stremlau et al. 2004).

The B-box is a distinct zinc binding sequence present on a number of developmentally important proteins (Torok and Etkin 2001) but the exact function of the B-box is unknown. The deletion or disruption of the B-box domain completely abolished HIV-1 restriction activity of rhesus TRIM5α, suggesting that this domain is essential for restriction activity (Javanbakht et al. 2005; Li et al. 2006a; Perez-Caballero et al. 2005a; Stremlau et al. 2004). Alteration of arginine 119 of human TRIM5α or the corresponding arginine 121 of rhesus TRIM5α diminished the abilities of the proteins to restrict retroviral infection and removal of the positively charged side chain from the B-box 2 arginines 119/121 resulted in diminished proteasome-independent turnover of TRIM5α (Diaz-Griffero et al. 2007b). A recent study by Sodroski's group suggests that the B-box 2 domain of TRIM5α promotes cooperative binding to the retroviral capsid by mediating higher-order self-association (Li and Sodroski 2008). Thus, the B-box domain of TRIM5α modulates capsid binding and retroviral restriction.

The coiled-coil region is composed of multiple alpha-helices involved in protein-protein interactions that may result in homo- or hetero-multimers (Meroni and Diez-Roux 2005; Nisole et al. 2005; Reymond et al. 2001). In vitro cross-linking studies demonstrate that the coiled-coil domain plays a critical role in oligomer formation of TRIM5α protein (Javanbakht et al. 2006b; Mische et al. 2005). It is proposed that oligomer formation allows the B30.2 domain of TRIM5α protein to be better positioned for binding to the target capsid. Consistent with this hypothesis, TRIM5α mutants lacking the coiled-coil domain fail to restrict viral infection (Javanbakht et al. 2006b; Perez-Caballero et al. 2005a).

The PRYSPRY domain, located at the C-terminus of TRIM5α protein, has a core composed of two β-sheets sandwiched together to form a central hydrophobic core and loops of variable length and containing non-conserved residues that protrude out from the core structure, based on comparisons with the molecular structures of

related proteins (Grutter et al. 2006; Masters et al. 2006; Ohkura et al. 2006; Woo et al. 2006). PRYSPRY domain was shown to be the determinant of the specificity of restriction (Nakayama et al. 2005; Ohkura et al. 2006; Perez-Caballero et al. 2005a; Stremlau et al. 2005; Yap et al. 2005). Substitution of R332 of human TRIM5α with a negatively-charged or non-charged amino acid is sufficient to allow restriction of HIV-1 without altering its ability to restrict N-MLV (Li et al. 2006b; Yap, et al. 2005). The regions of variability among TRIM5α PRYSPRY domains for different species are located on the protruding variable loops (Ohkura et al. 2006; Song et al. 2005b; Woo et al. 2006). Initial studies using chemical crosslinking suggested that TRIM5α may function as a trimer, but recent studies using purified recombinant TRIM5-21R, which contains the RING domain of TRIM21 in the backbone of TRIM5, by Sodroski's and Sundquist's groups suggest that TRIM5α forms stable dimers and recognizes retroviral capsids through direct interactions mediated by the PRYSPRY domain (Kar et al. 2008; Langelier et al. 2008).

4.3 How TRIM5α Works?

The viral determinant of susceptibility to TRIM5α-mediated restriction is the capsid protein (Cowan et al. 2002; Hatziioannou et al. 2004a; Owens et al. 2004; Owens et al. 2003; Towers et al. 2000). Restriction of retroviral infection by TRIM5α is saturated at high levels of input virions or virus-like particles and only the properly processed and assembled form of a condensed viral core is able to abrogate the restriction (Besnier et al. 2002; Cowan et al. 2002; Dodding et al. 2005; Forshey et al. 2005; Munk et al. 2002; Owens et al. 2004). Studies from several laboratories suggest that the PRYSPRY domain of TRIM5α is responsible for recognizing a conformational ligand on the viral capsid and that the RBCC domains provide an effector function by unknown mechanism (Javanbakht et al. 2006b; Li et al. 2006b; Owens et al. 2004; Perron et al. 2006, 2007; Sayah et al. 2004; Sebastian and Luban 2005; Stremlau et al. 2006a). Several possibilities can be envisioned for the possible mechanisms for TRIM5α restriction: it may bind and sequester the incoming virion core in a subcellular compartment; it may modify the virion core and target for degradation; it may interfere with normal uncoating; or it may inhibit trafficking of preintegration complex. Multiple mechanisms or pathways might be involved in TRIM5α restriction.

4.3.1 Inhibition of Normal Uncoating

In most instances, TRIM5α proteins impair retroviral infection early after entry into target cells, reducing the efficiency of reverse transcription (Keckesova et al. 2004; Perez-Caballero et al. 2005b; Stremlau et al. 2004), raising the possibility that TRIM5α might interfere with normal uncoating of the incoming viral cores.

Recently, it has been shown that TRIM5α binds to the restriction-sensitive retroviral capsid (Sebastian and Luban 2005; Stremlau et al. 2006a) and causes an accelerated uncoating (Stremlau et al. 2006a). Uncoating is a poorly understood process, and it is still uncertain whether it is an active process requiring energy and/or specific host cell components, or whether it occurs passively (Greber et al. 1994; Narayan and Young 2004). HIV-1 cores are relatively unstable in vitro and, in the infected cell the capsid protein is thought to undergo disassembly soon after virus entry (Forshey et al. 2002; Grewe et al. 1990). Analyses of the viral components of the HIV-1 reverse transcription or preintegration complexes failed to detect significant amounts of the capsid protein (Bukrinsky et al. 1993; Farnet and Haseltine 1991; Fassati and Goff 2001; Karageorgos et al. 1993; Miller et al. 1997). The analysis of HIV-1 Gag mutants suggests that capsid disassembly demonstrates precise requirements; both increases and decreases in capsid stability resulted in decreased HIV-1 replication ability (Forshey et al. 2002). Therefore, an accelerated or premature uncoating could account for one of the modes of TRIM5α-mediated HIV-1 restriction.

4.3.2 Ubiquitin Ligase Activity of TRIM5α

Protein ubiquitination and the subsequent degradation of ubiquitinated proteins by the proteasomal pathway are essential for a wide range of cellular functions (Freemont 2000). Proteasome-independent functions of protein ubiquitination are also involved in regulating a variety of protein functions including transport and processing (Schnell and Hicke 2003). Recently, a RING domain-dependent, auto-ubiquitination activity of TRIM5 protein has been demonstrated in vitro (Xu et al. 2003; Yamauchi et al. 2008). Then, an interesting question arises: what is the substrate of the TRIM5α enzyme other than itself? The identification of interaction partner or cofactor of TRIM5α will hopefully answer the question. Ubiquitination of the incoming HIV-1 cores could potentially lead to the degradation of the modified viral cores by proteasome system or could interfere with the trafficking of the modified viral cores. However, TRIM5α-mediated ubiquitination of HIV-1 cores has not so far been demonstrated.

4.3.3 Proteasome

It has been shown that disrupting proteasome function relieves rhesus TRIM5α restriction of HIV-1 late RT products even though 2-LTR circle production and viral infection remained blocked, suggesting some contribution of proteasome activity to TRIM5α activity (Anderson et al. 2006; Wu et al. 2006). Therefore, a two step process of TRIM5α restriction was proposed: TRIM5α acts prior to complete reverse transcription of viral RNA and may also inhibit trafficking of the preintegration complex. A recent study showed that treatment of cells with proteasome inhibitors prevented TRIM5α-dependent loss of particulate CA

protein (Diaz-Griffero et al. 2007a), indicating the potential involvement of proteasome activity in TRIM5α-induced virus uncoating. How much proteasome contributes to the restriction activity mediated by TRIM5α and its mechanism remain to be defined.

4.3.4 TRIM5α Turnover

Human and rhesus TRIM5α proteins stably expressed in HeLa cells were shown to be rapidly turned over, with half-lives of 50–60 min (Diaz-Griffero et al. 2006). The high rate of TRIM5α turnover creates opportunities for a rapid regulation of the levels of these proteins in response to viral infection or other stimuli. Both proteasome-dependent and proteasome-independent modes for the turnover of TRIM5α protein has been proposed (Diaz-Griffero et al. 2006, 2007b).

Recently, it was shown that TRIM5α is targeted for degradation by a proteasome-dependent mechanism following encounter of a restriction-sensitive retroviral core (Rold and Aiken 2008). This study proposed two potential outcomes of TRIM5α-CA interaction: (1) proteasomal degradation of a TRIM5α-CA complex, resulting in functional decapsidation of the viral core and a premature uncoating, and (2) dissociation of CA from the core followed by its release from TRIM5α, leading to destruction of the restriction factor and decapsidation of the core but not necessarily degradation of CA. It will be interesting to determine whether HIV-1-induced degradation of TRIM5α is dependent on the self-ubiquitination activity of TRIM5α or dependent on other host cell ubiquitin ligases.

4.3.5 TRIM5α dynamics and trafficking

It was shown that TRIM5α cytoplasmic bodies are highly mobile and use the microtubule network to navigate throughout the cytoplasm, and that TRIM5α proteins are dynamically exchanged between the cytoplasmic bodies and the diffuse cytoplasmic population (Campbell et al. 2007), suggesting a more active role of TRIM5α in antiviral activity. Furthermore, it was reported that there is a dynamic interaction between rhesus TRIM5α and HIV-1 viral complexes, including the de novo formation of TRIM5α cytoplasmic body-like structures around viral complexes (Campbell et al. 2008).

A previous study showed that heat shock proteins Hsp70 and Hsp90 colocalize with TRIM5α cytoplasmic bodies (Diaz-Griffero et al. 2006). Hsp70 and Hsp90 proteins are the components of molecular chaperones which play a critical function in protein folding by promoting and maintaining the native conformation of cellular proteins and in some cases in protein sorting (Young et al. 2004). It remains to be determined whether these molecular chaperones directly interact with TRIM5α and contribute to the turnover, trafficking, or the restriction activity of TRIM5α protein. Our understanding of the mechanism of HIV-1-restricting activity of TRIM5α may

depend on the complete understanding of the components and function of the TRIM5α complexes and the dissection of the interaction partners of TRIM5α.

4.3.6 Cyclophilin A

The CypA-CA interaction has been shown to assist HIV-1 replication in some human cells (Franke and Luban 1996; Hatziioannou et al. 2005; Sokolskaja et al. 2004; Thali et al. 1994). Initially, it was thought that CypA in producer cells plays an important role in HIV-1 replication. However, recent findings support that CypA is more important in target cells than in producer cells for HIV-1 replication (Kootstra et al. 2003; Towers et al. 2003). It has been hypothesized that human encode an unknown factor that can negatively affect HIV-1 replication, and CypA binding to HIV-1 CA can protect HIV-1 from this unknown factor (Sokolskaja, et al. 2006; Towers et al. 2003). In contrast to the positive effects of CypA on HIV-1 replication in human cells, CypA exerts negative effects on HIV-1 replication in Old World monkey cells because CypA-CA interactions sensitize HIV-1 to the restriction from Old World monkey TRIM5α proteins (Berthoux et al. 2005; Keckesova et al. 2006; Stremlau et al. 2006b).

CypA interacts with diverse lentiviral capsids including HIV-1, SIV_{cpz}, SIV_{agmTAN}, and FIV (Lin and Emerman 2006). It was proposed that CypA binding to HIV-1 CA induces the conformational change of viral core and renders HIV-1 CA more recognizable by the PRYSPRY domain of TRIM5α (Berthoux et al. 2005; Keckesova et al. 2006). However, HIV-1 variant (e.g., G89V), which does not bind CypA, is still susceptible to the TRIM5α restriction in a CypA-independent manner (Lin and Emerman 2008; Stremlau et al. 2006b). These findings support the idea that TRIM5α restriction of HIV-1 is composed of both CypA-dependent and CypA-independent components (Keckesova et al. 2006; Lin and Emerman 2008; Stremlau et al. 2006b). The ridge formed by helices 3 and 6 on CA has been reported to determine viral susceptibility to the TRIM5α restriction (Owens et al. 2004). A recent study showed that two loops on the HIV-1 capsid, one between the 4th and 5th helices (4–5 loop) and the other between the 6th and 7th helices (6–7 loop), are responsible for the HIV-1 susceptibility to the CypA-dependent TRIM5α restriction (Lin and Emerman 2008).

4.4 Polymorphism

An analysis of sequence data collected from HIV/AIDS cohorts, human genomic DNA diversity collections, and human SNP databases revealed polymorphism in TRIM5 (Goldschmidt et al. 2006; Javanbakht et al. 2006a; Sawyer et al. 2006; Speelmon et al. 2006; van Manen et al. 2008). These include residues in the RING domain (H43Y), in the B-box 2 domain (V112F), in or near the coiled-coil domain (R136Q, R238W, G249D), and in the PRYSPRY domain (H419Y). Two

polymorphisms in the TRIM5 gene (H43Y and R136Q) were shown to affect the antiviral activity of TRIM5α in vitro. For example, human TRIM5α with the H-to-Y change at position 43 showed a reduced ability to restrict N-MLV in tissue culture-based assays (Goldschmidt et al. 2006; Javanbakht et al. 2006a; Sawyer et al. 2006). For the residue at position 136, one study reported that R-to-Q change at position 136 rendered a slightly more effective restriction of HIV-1 (Javanbakht et al. 2006a), although a different study did not detect a difference (Goldschmidt et al. 2006). A recent study reported that an accelerated disease progression was observed for individuals who were homozygous for the 43Y genotype as compared to individuals who were heterozygous or homozygous for the 43H genotype (van Manen et al. 2008), suggesting that polymorphisms in the TRIM5 gene may influence the clinical course of HIV-1 infection.

5 Conclusion

The recent discovery of TRIM5α has revealed a complex interaction between the incoming virion and the host factor, influencing the postentry replication steps in the retroviral life cycle. TRIM5α reduces the accumulation of reverse transcriptase products possibly by interfering with the normal uncoating process. Little is known about the uncoating process, that occurs shortly after a retrovirus fuses with the cell membrane. There are many questions to be addressed to understand the mechanisms of TRIM5α restriction. Defining the capsid uncoating process and the cellular factors involved will be critical to understanding the mechanisms of TRIM5α restriction. The role of ubiquitin and TRIM5α E3 ubiquitin ligase activity needs to be determined. The availability of methods for producing and purifying TRIM5 derivatives should expedite studies of their structure and mechanism of action in restricting retroviral infection. Equally important is identifying TRIM5α-binding proteins or cofactors and understanding the normal function of TRIM5α. The elucidation of TRIM5 mechanism may facilitate pharmacological and genetic intervention to induce currently nonrestrictive human TRIM genes to target and restrict HIV-1.

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