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REVIEW ARTICLE

Restriction factors of retroviral replication: the example of Tripartite Motif (TRIM) protein 5α and 22

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Abstract Viral tropism, replication, and pathogenesis are determined by multiple interactions between the pathogen and the host. In the case of retroviruses, and in particular, the human immunodeficiency virus, the specific interaction of the envelope protein with the host receptors and co-receptors is essential to gain entry in the cells. After entry, the success of retroviruses to complete their life cycle depends on a complex interplay between the virus and host proteins. Indeed, the cell environment is endowed with a number of factors that actively block distinct stage(s) in the microbial life cycle. Among these restriction factors, Tripartite Motif-5α (TRIM5α) has been extensively studied; however, other TRIM family members have been demonstrated to be anti-retroviral effector proteins. This article reviews, in particular, the current knowledge on the anti-retroviral effects of TRIM5α and TRIM22.

Keywords Retroviral infection · Host restriction factors · TRIM family proteins

Introduction

The concept of retroviral restriction dates back to 1957, when Charlotte Friend observed that different strains of mice varied in their susceptibility to the Friend strain of murine leukemia virus (MLV), a member of the gamma-retroviral genus of the *Retroviridae* family of viruses (Friend 1957). Further genetic studies in mice revealed that virus susceptibility was determined by genetic traits termed "Friend virus susceptibility 1 (*Fv1*)" genes (Hartley et al. 1970; Lilly 1967; Pincus et al. 1971). Nearly 40 years after the first observation of a murine strain-specific block to MLV, *Fv1* was cloned (Best et al. 1996). From the analysis of mammalian species, however, no genes were found closely related to *Fv1* with the exception of mice (Besnier et al. 2003; Best et al. 1996). These findings were unexpected since a range of mammals, including humans, also demonstrated a specific resistance to MLV (Besnier et al. 2003; Towers et al. 2000). Thus, it has been suggested that human cells expressed the restriction factor 1 (Ref1) that inhibits MLV replication like the dominant Fv1 gene product (Towers et al. 2000). Furthermore, primate lentiviruses, including the human immunodeficiency virus type-1 (HIV-1) and the simian immunodeficiency virus (SIV), encounter a similar dominant restriction activity in many non-human primate cells attributed to a gene named lentivirus susceptibility factor 1 (Lv1) (Besnier et al. 2002; Cowan et al. 2002; Munk et al. 2002). The gene responsible for Lv1 activity was identified by introducing a cDNA expression library from HIV resistant Rhesus (Rh) macaque lung fibroblasts into HIV permissive human cells, and selecting for cells that expressed resistance to HIV-based vectors (Stremlau et al. 2004). The active Rh cDNA was found to encode the "alpha" spliced variant of Tripartite Motif 5α (TRIM5α), a member of a large family of proteins known as the TRIM proteins (Reymond et al. 2001). The expression of the Rh cDNA was sufficient to restrict incoming HIV-1, whereas its human ortholog was not. Afterwards, a number of groups demonstrated that the

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TRIM5α protein of different primates has a comparable antiretroviral activity against a variety of retroviruses (Hatziioannou et al. 2004; Keckesova et al. 2004; Perron et al. 2004; Yap et al. 2004). These findings suggest that primate TRIM5α proteins provide a general defense mechanism against retroviral infection.

TRIM protein structure

The members of the TRIM family were originally defined as proteins that contain Really New Interesting Protein (RING), either 1 or 2 B-boxes, and coiled-coil domains (RBCC) (Borden 1998; Reddy et al. 1992). The RBCC motif, located at the N-terminus, is well conserved among TRIM proteins, whereas the C-terminal domains vary. The C-terminal domains determine the classification of the 72 human TRIM proteins in 11 subfamilies while one group (including TRIM14 and TRIM20) remains unclassified due to the lack of a RING domain (Nisole et al. 2005; Ozato et al. 2008; Sardiello et al. 2008; Short and Cox 2006; Carthagena et al. 2009). Among others, the subfamily C-IV encompasses several members with anti-viral activity (Ozato et al. 2008). This subfamily is characterized by a SPRY domain in combination with a PRY domain to form a so-called B30.2 domain located at the C-terminus immediately following the RBCC motif (see Fig. 1). The B30.2 domain was originally identified as a protein domain encoded by a single exon (called B30.2) in the human major histocompatibility complex (MHC) class I region (Meyer et al. 2003), while the SPRY domain was identified as a conserved domain in the non-receptor tyrosine kinase spore lysis A of *Dictyostelium discoideum* and in mammalian ryanodine receptors (Ponting et al. 1997). The PRY domain was named for conserved sequences, located N-terminally to SPRY domains that appear to have arisen

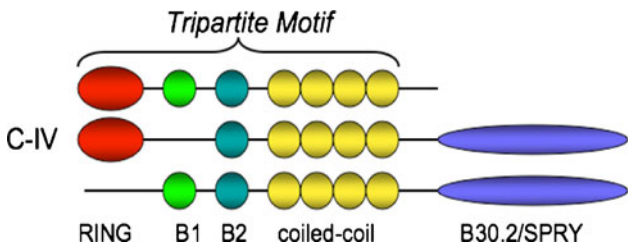

Fig. 1 Schematic structure of the domains present in the C-IV class of TRIM/RBCC family. The TRIM/RBCC proteins share RING, B1, B2 and coiled-coil domains whereas the C-terminals are variable. The C-terminal domains determine the classification of the 72 human TRIM proteins in 11 subfamilies while one group (including TRIM14 and TRIM20) remains unclassified due to the lack of a RING domain (Carthagena et al. 2009). The C-IV subfamily that includes the TRIM5 and TRIM22 members is characterized by the presence of the only B-box 2

relatively recently during evolution, distinguishing the B30.2 domain from the more ancient SPRY domain (Rhodes et al. 2005; Woo et al. 2006a, b).

About their structure, TRIM proteins form high-molecular-mass complexes that localize to specific subcellular compartments present either in the cytoplasm or in the nucleus (Reymond et al. 2001). For example, TRIM19, also known as Promyelocytic Leukemia (PML) protein, exists in subnuclear structures known as "nuclear bodies", whereas TRIM5 is normally located in distinct cytoplasmic aggregates designated as "cytoplasmatic bodies". These bodies are mobile, highly dynamic structures that turn over rapidly due to exchange of protein between the nucleus and the cytoplasm (Campbell et al. 2007). The formation of cytoplasmic bodies, however, is not a requirement for retroviral restriction (Perez-Caballero et al. 2005a; Song et al. 2005).

TRIM protein function

TRIM family proteins have been involved in a variety of cellular processes, such as signal transduction, transcriptional regulation, cell proliferation, oncogenesis, and apoptosis (Meroni and Diez-Roux 2005; Ozato et al. 2008). Furthermore, a number of TRIM proteins have been found to interfere with the replication cycle of different viruses (see Table 1). TRIM19, for example, has been implicated in the regulation of infection by a variety of RNA and DNA viruses, including adenoviruses, herpes simplex viruses (HSV) and human cytomegalovirus (CMV) (Everett and Chelbi-Alix 2007). These findings, together with the observation that the expression of many TRIM proteins is induced by interferons (IFNs) (Carthagena et al. 2009), suggest that they likely represent important mediators of innate immunity. In this regard, it has been recently shown that TRIM21 plays a negative role in the regulation of the NF-kB dependent pro-inflammatory cytokine response in mice (Yoshimi et al. 2009). Another member, i.e. TRIM30, inhibits the Toll-like receptor (TLR) signaling by degrading TAK1-binding protein (TAB) 2 and 3 and it contributes to the inhibition of TLR-mediated NF-kB activation (Shi et al. 2008).

In regard to retroviruses, multiple TRIM proteins beside TRIM5 display antiviral activities against a panel of distantly related retroviruses when exogenously expressed (see Table 1) (Li et al. 2007; Uchil et al. 2008; Zhang et al. 2006). For example, mouse TRIM28 was shown to restrict MLV replication by promoting gene silencing (Wolf and Goff 2007). In particular, TRIM28 functions as a transcriptional co-repressor recruiting several other factors involved in transcriptional silencing and heterochromatin formation (Le Douarin et al. 1996; Schultz et al. 2002).

Table 1 Role of TRIM family members in viral infections

Member	Virus(es)	Viral target(s)	Note(s)	Key references
TRIM1	MLV	CA	Restriction activity	Yap et al. (2004), Zhang et al. (2006)
TRIM5	HIV, MLV, SIV, FIV, EIAV	CA	Restriction activity	Stremlau et al. (2004), Keckesova et al. (2004), Yap et al. (2004)
TRIM6	HIV	CA	No restriction activity	Li et al. (2007)
TRIM8	HIV, MLV	Unknown	Inhibition of entry, inhibition of MLV particle release	Uchil et al. (2008)
TRIM9	Unknown	Unknown	Up-regulated in FcgammaR-activated macrophages	Carthagena et al. (2009)
TRIM10	HIV	Unknown	Inhibition of entry	Uchil et al. (2008)
TRIM11	HIV, MLV	Unknown	Inhibition of HIV entry, particle production and release. Enhancement of MLV entry	Uchil et al. (2008)
TRIM13	MLV	Unknown	Inhibition of particle production and release	Uchil et al. (2008)
TRIM14	HIV, MLV	Unknown	Inhibition of particle production and release	Uchil et al. (2008)
TRIM15	HIV, MLV	Unknown	Inhibition of particle release	Uchil et al. (2008)
TRIM19	MLV, VSV, Influenza A, CMV, HSV-1, Ebola and Lassa viruses, LCMV, human foamy virus, adenovirus, HIV	LCMV Z protein, HCV core protein	Inhibition of p53, inhibition of particle release	Everett and Chelbi-Alix (2007), Turelli et al. (2001), Uchil et al. (2008)
TRIM21	HIV	Unknown	Enhancement of entry	Uchil et al. (2008)
TRIM22	HIV	LTR and Gag	Inhibition of transcription, assembly and release	Tissot and Mechti (1995), Barr et al. (2008)
TRIM25	MLV, Sendai virus, New Castle disease virus, VSV	Unknown	Ubiquitination of retinoic acid-inducible gene I product (RIG-I)	Gack et al. (2007), Uchil et al. (2008)
			Inhibition of entry and particle release	
TRIM26	HIV, MLV	Unknown	Inhibition of entry and particle release	Uchil et al. (2008)
TRIM28	MLV, HTLV-1	PBS	Inhibition of transcription and particle release	Wolf and Goff (2007), Uchil et al. (2008)
TRIM30	MLV	Unknown	Enhancement of entry	Uchil et al. (2008)
TRIM31	HIV	Unknown	Inhibition of entry	Uchil et al. (2008)
TRIM32	HIV-1, HIV-2 and EIAV	Tat	Transcription regulation	Fridell et al. (1995)
TRIM34	SIV	CA	Weak restriction activity	Li et al. (2007), Zhang et al. (2006)
TRIM35	MLV	Unknown	Inhibition of particle release	Uchil et al. (2008)
TRIM38	HIV	Unknown	Enhancement of entry	Uchil et al. (2008)
TRIM54	Unknown	Unknown	Up-regulated in FcgammaR-activated macrophages	Carthagena et al. (2009)
TRIM56	HIV	Unknown	Inhibition of entry	Uchil et al. (2008)
TRIM62	MLV	Unknown	Inhibition of entry	Uchil et al. (2008)

EIAV Equine infectious anemia virus, *VSV* vesicular stomatitis virus, *LCMV* lymphocytic choriomeningitis virus, *R* receptor, *CA* capsid, *LTR* long terminal repeat, *PBS* primer binding site

TRIM28-mediated MLV restriction is effective in embryonic stem cells since this TRIM protein is part of a nuclear repressor complex that binds to the retroviral "Primer Binding Site (PBS)". In the case of MLV, PBS is a sequence complementary to the cellular tRNA for proline ($tRNA^{Pro}$) that is used to prime minus-strand DNA synthesis during reverse transcription (Harada et al. 1979). However, the PBS overlaps with a so-called "Repressor-Binding Site (RBS)" (Wolf and Goff 2007). Since TRIM28, in spite of being recruited to different PBS including the tRNA of Lysine 1 and 2 (Wolf et al. 2008), does not bind to DNA, other factors must recruit TRIM28

to mediate the specific silencing induced by different DNA-binding proteins. In this regard, Wolf and Goff (2009) have recently identified a novel zinc finger protein, ZFP208, that functions as a recognition molecule bridging the integrated proviral DNA with TRIM28. Expression of ZFP208 is sufficient to render even differentiated cells resistant to MLV infection. In addition, ZFP208 potently inhibits transcription from DNA constructs of the human T cell lymphotropic virus-1 (HTLV-1) that utilizes the same primer tRNA (Wolf and Goff 2009). Thus, this novel zinc finger protein is a new DNA binding factor that specifically recognizes a large subset of mammalian retroviruses and retroelements and targets them for transcriptional silencing. This breakthrough observation suggests that ZFP809 has evolved as a stem cell-specific retroviral restriction factor constituting a novel component of the intrinsic defense of stem cells.

TRIM5α

In spite of the presence of 72 family members, only a few proteins have been extensively studied and, among others, TRIM5α. Indeed, TRIM5 possesses multiple spliced isoforms, each characterized by an alternative C-terminus (see Fig. 2). The longest isoform, TRIM5α, is the only one that encodes a C-terminal B30.2 domain and exhibits antiviral activity (Stremlau et al. 2004). The shorter isoforms, TRIM5γ and TRIM5δ, lack the C-terminus and, when overexpressed, act in a dominant negative fashion on

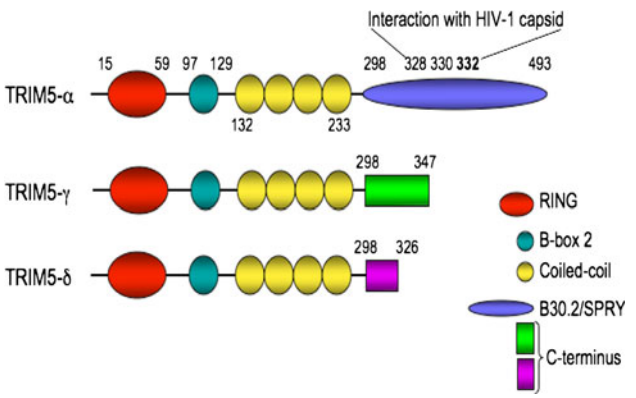

Fig. 2 Schematic representation of the main splice variants of TRIM5. The TRIM5 gene is endowed with multiple isoforms that are characterized by alternative C-terminus. The TRIM5α possesses the B30.2/SPRY domain whereas the TRIMγ and TRIMδ undergo alternative splicing within the coding region resulting in a frameshift and use of upstream termination codons. The B30.2/SPRY domain of TRIM5α contains the amino acids critical for the interaction with the HIV-1 capsid. In particular, the amino acid at position 332 accounts for full HIV-1 restriction in human TRIM5α (arginine) when it is replaced with that of the rhesus TRIM5α protein (proline) (Stremlau et al. 2005; Yap et al. 2005)

TRIM5α targets therefore promoting the rescue of restricted infection (Passerini et al. 2006; Stremlau et al. 2004). TRIM5α is a protein responsible for the species-specific restriction of HIV entry via binding to incoming retroviral capsid (CA) protein mediated by its C-terminal B30.2 domain causing premature disassembly of the CA (Sawyer et al. 2005; Stremlau et al. 2005; Yap et al. 2005). In addition, TRIM5α has been postulated to interfere with late events in the viral life cycle affecting particle release by promoting the degradation of nascent Gag (Sakuma et al. 2007). This interpretation, however, has been debated since it requires high levels of TRIM5α expression (Zhang et al. 2008). Indeed, the precise mechanism of restriction mediated by TRIM5α remains still unknown, although the role of the B30.2 domain of TRIM5α in restricting retroviruses became apparent in early experiments in which Rh and hu TRIM5α were shown to directly interact with the CA protein of HIV-1 and MLV, respectively (Sebastian and Luban 2005; Stremlau et al. 2006). The B30.2 domain is required for binding CA and restriction, as demonstrated by the observation that both deletion mutants and naturally occurring isoforms of Rh TRIM5α, lacking the B30.2 domain fail to restrict HIV-1 (Stremlau et al. 2004, 2006). Furthermore, the replacement of segments in the B30.2 variable region of hu TRIM5, which fails to restrict HIV-1, with analogous sections from Rh TRIM5α, has demonstrated restriction activity against HIV-1 (Stremlau et al. 2005). Additional mutagenesis experiments have shown that a single amino acid change in hu TRIM5α (R332P), matching the analogous residue in the B30.2 variable region of Rh TRIM5α, confers antiviral activity against HIV-1 (Fig. 2) (Perron et al. 2006; Stremlau et al. 2005; Yap et al. 2005). In this regard, it has recently been shown by experiments of site-directed mutagenesis of 9 clusters of 3 charged amino acids in the conserved regions of the B30.2 domain that retroviral restriction is dependent on the interaction of TRIM5α with CA (Sebastian et al. 2009). Furthermore, a highly conserved surface patch on the TRIM5α B30.2 domain has been described as crucial for the restriction of the incoming virions independently of CA binding (Sebastian et al. 2009).

By analyzing TRIM5α orthologous sequences, it has been proposed that the TRIM5 gene is under positive purifying selection in that the ratio between non-synonymous coding mutations (dN) and synonymous non-coding mutations (dS) is >1.0 (Sawyer et al. 2005). In particular, a variable region of the B30.2 domain was predicted to be under positive selection (Sawyer et al. 2005). Intriguingly, this variable region lies within the PRY domain (amino acids 298–355) of the B30.2 domain, a relatively recent addition to the older SPRY domain since B30.2 domains are only found in vertebrates (Sawyer et al. 2005). These observations support the notion that a positive selective

pressure during evolution led to a species-specific block of retroviral infections and that the B30.2 domain of TRIM5α is a determinant of a species-specific retroviral tropism.

The importance of TRIM5α in retroviral infection is highlighted by a retrotransposition event that occurred in Owl monkeys (*Aotus trivirgatus*) leading to the generation of an ortholog of TRIM5α that blocks HIV-1 infection in this species. The retrotransposition event deleted the B30.2 domain of TRIM5α and replaced it by the coding sequence of Cyclophilin A (CypA), a well known target of immunosuppressive drugs, such as Cyclosporine A (CsA), leading to a fusion protein termed TRIMCyp (Sayah et al. 2004). This recombination event occurred independently in Pig tailed (Pgt) macaques that express another variant of TRIMCyp with a distinct specificity in that PgtTRIMCyp restricts the Feline Immunodeficiency Virus (FIV), a retrovirus causing an AIDS-like syndrome in cats (Gardner and Luciw 1989), but not HIV (Virgen et al. 2008). Furthermore, TRIMCyp was discovered as restriction factor for HIV-2 infection in Rhesus macaque (Wilson et al. 2008). CypA is a ubiquitously expressed peptidylprolyl isomerase that was earlier shown to interact with the CA protein of HIV-1, but not of HIV-2 (Luban et al. 1993). Disruption of the interaction between CypA and HIV-1 CA by competitive inhibitors such as CsA, by mutations in the CA protein (G89V or P90A), or by suppression of CypA expression demonstrated that HIV-1 infection of human cells is highly dependent on CA interaction with CypA (Berthoux et al. 2005; Franke et al. 1994; Luban et al. 1993). Likely, CypA confers protection to the incoming virus from a yet unidentified restriction factor (Sokolskaja et al. 2006). In non-human primates, CypA does not bind to CA (Braaten et al. 1996; Lin and Emerman 2006); however, when CypA is fused to TRIM5, it restricts HIV-1 in the same way the B30.2 does for TRIM5α. Thus, CypA functions in an opposite manner in non-human primate cells as compared to human cells by promoting restriction rather than replication (Luban 2007).

The role of the N-terminus RBCC domain of TRIM5α in retroviral restriction has also been studied extensively. The RING domain of some TRIM, also found in proteins not belonging to the TRIM family, possesses an E3 ligase activity involved in the addition of ubiquitin to proteins targeting them for proteasomal degradation (Joazeiro et al. 1999; Lorick et al. 1999; Boutell and Everett 2003; Jones and Gellert 2003; Jang 2004; Vichi et al. 2005; Yamauchi et al. 2008). This observation has led to the hypothesis that the destruction of HIV-1 CA could be ubiquitin-mediated although independent studies have shown that proteosomal degradation is not required for TRIM5α mediated restriction (Perez-Caballero et al. 2005b). Even in the case of HIV-1 restriction induced by TRIMCyp, the rapid kinetics of CA inactivation occurred independently of cytoplasmic bodies, ubiquitin, and proteasome activity (Perez-Caballero et al. 2005b). Among the RBCC components that contribute to HIV-1 restriction, the B-box 2 plays a crucial role, since a loss of activity was caused by its deletion (Diaz-Griffero et al. 2007; Javanbakht et al. 2005; Perez-Caballero et al. 2005a). Indeed, the B-box and coiled-coil motifs have been implicated in mediating protein–protein interactions, either with the same or other proteins to form multiprotein complexes (Mische et al. 2005). Thus, the RBCC domain seems to act as a scaffold on which other cellular components of the restriction machinery assemble, possibly leading to the sequestering of incoming viral particles. The RBCC has also been implicated in the cellular localization of the TRIM proteins (Reymond et al. 2001), guiding the restriction factor into the appropriate cellular compartment utilized by the incoming virus. Hence, another role of the RBCC in restriction could be to form higher order TRIM5α arrays that contribute to CA binding avidity, as recently suggested (Diaz-Griffero et al. 2009).

Despite the molecular characterization of the mechanism of TRIM5 restriction, the influence of human TRIM5 in the HIV-1 infection in vivo remains largely unexplored. In this regard, a recent report has shown the expression of hu TRIM5α in the peripheral blood mononuclear cells obtained from the CAPRISA cohort study of sex workers at high risk for infection by real time PCR (Sewram et al. 2009). Notably, HIV-negative individuals had statistically significant higher levels of TRIM5α as compared to HIV-positive individuals. In addition, these lower levels of TRIM5α expression were most likely the result of seroconverters having significantly lower hu TRIM5α levels at baseline (before infection), compared with nonseroconverters (Sewram et al. 2009). Consequently, the levels of expression and role of TRIM5α and other endogenous TRIMs mediating "intrinsic immunity" to HIV will need to be thoroughly evaluated at the sexual mucosal level, a primary site of viral transmission.

TRIM22

TRIM22, previously described as "Stimulated Trans-acting Factor of 50 kDa (Staf50)", is located on chromosome 11 adjacent to TRIM5 (Sawyer et al. 2007; Tissot et al. 1996). TRIM22 was originally identified in a screening for IFN-inducible genes in the human B lymphoblastoid Daudi cell line (Tissot and Mechti 1995). In the same study, co-transfection of COS-7 cells with a TRIM22 expression plasmid inhibited an LTR-mediated expression of a reporter gene suggesting a potential role of TRIM22 in controlling HIV transcription (Tissot and Mechti 1995). No information, however, were provided concerning its mechanism of action. During a screening of gene expression in monocytic

cell lines, type I and II IFN and other stimuli, in particular, bacterial lipopolysaccharide (LPS) also induced TRIM22 (Bouazzaoui et al. 2006). Indeed, overexpression of TRIM22 inhibited HIV-1 infection and replication in primary human monocyte-derived macrophages (MDM), a relevant target of HIV-1 infection, although no precise mechanism of action was defined in this study (Bouazzaoui et al. 2006). Interference of TRIM22 on late steps of the HIV life cycle has also been proposed based on the observation that p24 Gag antigen levels were decreased in the supernatant of infected MDM after either transient transfection or transduction with TRIM22 expressing vectors (Bouazzaoui et al. 2006). In support of this hypothesis, a subsequent study showed that IFN-induction of TRIM22 interferes with the late steps of the HIV life cycle at the levels of virion assembly and release thereby inhibiting the accumulation of viral particles in the supernatant of various cell lines such as HOS, U2OS 143B and HeLa cells (Barr et al. 2008). In particular, an inhibitory effect on Gag synthesis was clearly shown in U2OS and 143B cell lines although not in HeLa cells (Barr et al. 2008), suggesting a role of TRIM22 in the assembly and release of virions rather than interference with Gag synthesis or viral transcription. The same study showed evidence of a direct interaction of TRIM22 with Gag polyprotein while a double mutant substituting the 2 cysteines at position 15 and 18 with alanine in the RING domain abolished TRIM22 effect on HIV particle release; however, the interaction between Gag and TRIM22 did not affect Gag protein stability and no ubiquitination of Gag was reported by effect of TRIM22 (Barr et al. 2008). Thus far, the substrate(s) of TRIM22 remains unknown in contrast to other members of the TRIM family, such as TRIM21 and TRIM25 that function as E3 ubiquitin ligase for "IFN regulating factor 8 (IRF-8)" and "Retinoic acid inducible gene I (RIG-1)", respectively (Oshiumi et al. 2009; Yoshimi et al. 2009). The mechanism of TRIM22 restriction on the late events of the HIV life cycle remains undefined and, nonetheless, it should be related to TRIM22 subcellular localization. In this regard, TRIM22 has been reported to reside either in the cytoplasm (Herr et al. 2009; Meroni and Diez-Roux 2005) or in the nucleus (Gao et al. 2009; Sivaramakrishnan et al. 2009a, b) likely accomplishing diverse biological effects. When TRIM22 was coupled to the green fluorescence protein (GFP), its presence was localized in cytoplasmic bodies in the U2OS cells (Meroni and Diez-Roux 2005) whereas an independent study of TRIM22-GFP fusion protein described a diffuse accumulation surrounding the nucleus of COS-7 cells (Herr et al. 2009). The same localization was observed in HeLa cells expressing endogenous TRIM22 (Herr et al. 2009). In contrast, a c-myc-tagged protein product linked to TRIM22 coding sequence amplified from human peripheral blood mononuclear cells localized TRIM22 exclusively in the nucleus (Duan et al. 2008). Furthermore, recent reports have shown that endogenous expression of TRIM22 occurred selectively in the nucleus of HeLa cells (Sivaramakrishnan et al. 2009a, b).

The formation of the nuclear bodies is dependent on TRIM22 B30.2/SPRY domain, although it has been reported that both a deletion mutant of the RING domain and a cysteine mutant in position 15 of the RING domain disrupt the nuclear bodies in HepG2 cells (Gao et al. 2009). Interestingly, nuclear bodies undergo dynamic changes during cell cycle progression (Sivaramakrishnan et al. 2009b) and TRIM22 forms nuclear bodies in G_0/G_1-phase in HeLa cells evolving into speckle-like structures during the S and probably G_2/M-phases (Sivaramakrishnan et al. 2009b).

In addition to HIV-1, TRIM22 has been shown to have suppressive activities on other viruses. In particular, the TRIM22 ubiquitin ligase activity seems to be required for the anti-viral activity against the encephalomyocarditis virus, a picornavirus that causes disease in mice (Eldin et al. 2009). More recently, TRIM22 has been shown to inhibit the replication of Hepatitis B virus (HBV) by interference with the HBV core promoter (Gao et al. 2009).

Beyond its anti-viral effects, TRIM22 has been involved in cell proliferation and survival and is a target of p53 (Obad et al. 2004). In this regard, promonocytic U937 cells devoid of endogenous p53 expression were electroporated with an expression vector encoding TRIM22 and the interference with cell clonogenic growth was monitored by colony formation in soft agar. TRIM22 reduced the capacity of these U937 cells to form colonies in soft agar consequently to their proliferation arrest and apoptosis (Obad et al. 2004). The same authors observed a differentiation stage-specific inverse correlation between the levels of TRIM22 expression and erythroid or granulocytic differentiation in CD34(+) human bone marrow progenitor cells (Obad et al. 2007).

Conclusions

The TRIM family is made by a list of 72 genes among which only a few have been studied in detail. The discovery of TRIM5α restriction of HIV-1 replication in monkey cells has shed light on the identification of restriction factors described 50 years ago. Several questions, however, remain open and the exact mechanism of TRIM5α or TRIM22 block of infectivity has not been defined. The identification of the precise viral and cellular partners will guide to the discovery of drugs that either mimic or enhance the effects of these restriction factors. Several other questions still remain open, particularly in regard with expression levels and inducibility of TRIM

family members in HIV-infected individuals during HIV disease progression and in response to antiretroviral agents.

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References

Barr SD, Smiley JR, Bushman FD (2008) The interferon response inhibits HIV particle production by induction of TRIM22. PLoS Pathog 4:e1000007

Berthoux L, Sebastian S, Sokolskaja E, Luban J (2005) Cyclophilin A is required for TRIM5{alpha}-mediated resistance to HIV-1 in Old World monkey cells. Proc Natl Acad Sci USA 102:14849–14853

Besnier C, Takeuchi Y, Towers G (2002) Restriction of lentivirus in monkeys. Proc Natl Acad Sci USA 99:11920–11925

Besnier C, Ylinen L, Strange B, Lister A, Takeuchi Y, Goff SP, Towers GJ (2003) Characterization of murine leukemia virus restriction in mammals. J Virol 77:13403–13406

Best S, Le Tissier P, Towers G, Stoye JP (1996) Positional cloning of the mouse retrovirus restriction gene Fv1. Nature 382:826–829

Borden KL (1998) RING fingers and B-boxes: zinc-binding protein-protein interaction domains. Biochem Cell Biol 76:351–358

Bouazzaoui A, Kreutz M, Eisert V, Dinauer N, Heinzelmann A, Hallenberger S, Strayle J, Walker R, Rubsamen-Waigmann H, Andreesen R, von Briesen H (2006) Stimulated trans-acting factor of 50 kDa (Staf50) inhibits HIV-1 replication in human monocyte-derived macrophages. Virology 356:79–94

Boutell C, Everett RD (2003) The herpes simplex virus type 1 (HSV-1) regulatory protein ICP0 interacts with and Ubiquitinates p53. J Biol Chem 278:36596–36602

Braaten D, Franke EK, Luban J (1996) Cyclophilin A is required for an early step in the life cycle of human immunodeficiency virus type 1 before the initiation of reverse transcription. J Virol 70:3551–3560

Campbell EM, Dodding MP, Yap MW, Wu X, Gallois-Montbrun S, Malim MH, Stoye JP, Hope TJ (2007) TRIM5 alpha cytoplasmic bodies are highly dynamic structures. Mol Biol Cell 18:2102–2111

Carthagena L, Bergamaschi A, Luna JM, David A, Uchil PD, Margottin-Goguet F, Mothes W, Hazan U, Transy C, Pancino G, Nisole S (2009) Human TRIM gene expression in response to interferons. PLoS One 4:e4894

Cowan S, Hatziioannou T, Cunningham T, Muesing MA, Gottlinger HG, Bieniasz PD (2002) Cellular inhibitors with Fv1-like activity restrict human and simian immunodeficiency virus tropism. Proc Natl Acad Sci USA 99:11914–11919

Diaz-Griffero F, Kar A, Perron M, Xiang SH, Javanbakht H, Li X, Sodroski J (2007) Modulation of retroviral restriction and proteasome inhibitor-resistant turnover by changes in the TRIM5alpha B-box 2 domain. J Virol 81:10362–10378

Diaz-Griffero F, Qin XR, Hayashi F, Kigawa T, Finzi A, Sarnak Z, Lienlaf M, Yokoyama S, Sodroski J (2009) A B-box 2 surface patch important for TRIM5{alpha} self-association, capsid-binding avidity and retrovirus restriction. J Virol 83:10737–10751

Duan Z, Gao B, Xu W, Xiong S (2008) Identification of TRIM22 as a RING finger E3 ubiquitin ligase. Biochem Biophys Res Commun 374:502–506

Eldin P, Papon L, Oteiza A, Brocchi E, Lawson TG, Mechti N (2009) TRIM22 E3 ubiquitin ligase activity is required to mediate antiviral activity against encephalomyocarditis virus. J Gen Virol 90:536–545

Everett RD, Chelbi-Alix MK (2007) PML and PML nuclear bodies: implications in antiviral defence. Biochimie 89:819–830

Franke EK, Yuan HE, Luban J (1994) Specific incorporation of cyclophilin A into HIV-1 virions. Nature 372:359–362

Fridell RA, Harding LS, Bogerd HP, Cullen BR (1995) Identification of a novel human zinc finger protein that specifically interacts with the activation domain of lentiviral Tat proteins. Virology 209:347–357

Friend C (1957) Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. J Exp Med 105:307–318

Gack MU, Shin YC, Joo CH, Urano T, Liang C, Sun L, Takeuchi O, Akira S, Chen Z, Inoue S, Jung JU (2007) TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. Nature 446:916–920

Gao B, Duan Z, Xu W, Xiong S (2009) Tripartite motif-containing 22 inhibits the activity of hepatitis B virus core promoter, which is dependent on nuclear-located RING domain. Hepatology 50:424–433

Gardner MB, Luciw PA (1989) Animal models of AIDS. Faseb J 3:2593–2606

Harada F, Peters GG, Dahlberg JE (1979) The primer tRNA for Moloney murine leukemia virus DNA synthesis. Nucleotide sequence and aminoacylation of tRNAPro. J Biol Chem 254:10979–10985

Hartley JW, Rowe WP, Huebner RJ (1970) Host-range restrictions of murine leukemia viruses in mouse embryo cell cultures. J Virol 5:221–225

Hatziioannou T, Perez-Caballero D, Yang A, Cowan S, Bieniasz PD (2004) Retrovirus resistance factors Ref1 and Lv1 are species-specific variants of TRIM5alpha. Proc Natl Acad Sci USA 101:10774–10779

Herr AM, Dressel R, Walter L (2009) Different subcellular localisations of TRIM22 suggest species-specific function. Immunogenetics 61:271–280

Jang JH (2004) FIGC, a novel FGF-induced ubiquitin-protein ligase in gastric cancers. FEBS Lett 578:21–25

Javanbakht H, Diaz-Griffero F, Stremlau M, Si Z, Sodroski J (2005) The contribution of RING and B-box 2 domains to retroviral restriction mediated by monkey TRIM5alpha. J Biol Chem 280:26933–26940

Joazeiro CA, Wing SS, Huang H, Leverson JD, Hunter T, Liu YC (1999) The tyrosine kinase negative regulator c-Cbl as a RING-type, E2-dependent ubiquitin-protein ligase. Science 286:309–312

Jones JM, Gellert M (2003) Autoubiquitylation of the V(D)J recombinase protein RAG1. Proc Natl Acad Sci USA 100:15446–15451

Keckesova Z, Ylinen LM, Towers GJ (2004) The human and African green monkey TRIM5alpha genes encode Ref1 and Lv1 retroviral restriction factor activities. Proc Natl Acad Sci USA 101:10780–10785

Le Douarin B, Nielsen AL, Garnier JM, Ichinose H, Jeanmougin F, Losson R, Chambon P (1996) A possible involvement of TIF1 alpha and TIF1 beta in the epigenetic control of transcription by nuclear receptors. EMBO J 15:6701–6715

Li X, Gold B, O'HUigin C, Diaz-Griffero F, Song B, Si Z, Li Y, Yuan W, Stremlau M, Mische C, Javanbakht H, Scally M, Winkler C, Dean M, Sodroski J (2007) Unique features of TRIM5alpha among closely related human TRIM family members. Virology 360:419–433

Lilly F (1967) Susceptibility to two strains of Friend leukemia virus in mice. Science 155:461–462

Lin TY, Emerman M (2006) Cyclophilin A interacts with diverse lentiviral capsids. Retrovirology 3:70

Lorick KL, Jensen JP, Fang S, Ong AM, Hatakeyama S, Weissman AM (1999) RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. Proc Natl Acad Sci USA 96:11364–11369

Luban J (2007) Cyclophilin A, TRIM5, and resistance to human immunodeficiency virus type 1 infection. J Virol 81:1054–1061

Luban J, Bossolt KL, Franke EK, Kalpana GV, Goff SP (1993) Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. Cell 73:1067–1078

Meroni G, Diez-Roux G (2005) TRIM/RBCC, a novel class of 'single protein RING finger' E3 ubiquitin ligases. Bioessays 27:1147–1157

Meyer M, Gaudieri S, Rhodes DA, Trowsdale J (2003) Cluster of TRIM genes in the human MHC class I region sharing the B30.2 domain. Tissue Antigens 61:63–71

Mische CC, Javanbakht H, Song B, Diaz-Griffero F, Stremlau M, Strack B, Si Z, Sodroski J (2005) Retroviral restriction factor TRIM5alpha is a trimer. J Virol 79:14446–14450

Munk C, Brandt SM, Lucero G, Landau NR (2002) A dominant block to HIV-1 replication at reverse transcription in simian cells. Proc Natl Acad Sci USA 99:13843–13848

Nisole S, Stoye JP, Saib A (2005) TRIM family proteins: retroviral restriction and antiviral defence. Nat Rev Microbiol 3:799–808

Obad S, Brunnstrom H, Vallon-Christersson J, Borg A, Drott K, Gullberg U (2004) Staf50 is a novel p53 target gene conferring reduced clonogenic growth of leukemic U-937 cells. Oncogene 23:4050–4059

Obad S, Olofsson T, Mechti N, Gullberg U, Drott K (2007) Expression of the IFN-inducible p53-target gene TRIM22 is down-regulated during erythroid differentiation of human bone marrow. Leuk Res 31:995–1001

Oshiumi H, Matsumoto M, Hatakeyama S, Seya T (2009) Riplet/RNF135, a RING finger protein, ubiquitinates RIG-I to promote interferon-beta induction during the early phase of viral infection. J Biol Chem 284:807–817

Ozato K, Shin DM, Chang TH, Morse HC 3rd (2008) TRIM family proteins and their emerging roles in innate immunity. Nat Rev Immunol 8:849–860

Passerini LD, Keckesova Z, Towers GJ (2006) Retroviral restriction factors Fv1 and TRIM5alpha act independently and can compete for incoming virus before reverse transcription. J Virol 80:2100–2105

Perez-Caballero D, Hatziioannou T, Yang A, Cowan S, Bieniasz PD (2005a) Human tripartite motif 5alpha domains responsible for retrovirus restriction activity and specificity. J Virol 79:8969–8978

Perez-Caballero D, Hatziioannou T, Zhang F, Cowan S, Bieniasz PD (2005b) Restriction of human immunodeficiency virus type 1 by TRIM-CypA occurs with rapid kinetics and independently of cytoplasmic bodies, ubiquitin, and proteasome activity. J Virol 79:15567–15572

Perron MJ, Stremlau M, Song B, Ulm W, Mulligan RC, Sodroski J (2004) TRIM5alpha mediates the postentry block to N-tropic murine leukemia viruses in human cells. Proc Natl Acad Sci USA 101:11827–11832

Perron MJ, Stremlau M, Sodroski J (2006) Two surface-exposed elements of the B30.2/SPRY domain as potency determinants of N-tropic murine leukemia virus restriction by human TRIM5alpha. J Virol 80:5631–5636

Pincus T, Rowe WP, Lilly F (1971) A major genetic locus affecting resistance to infection with murine leukemia viruses. II. Apparent identity to a major locus described for resistance to friend murine leukemia virus. J Exp Med 133:1234–1241

Ponting C, Schultz J, Bork P (1997) SPRY domains in ryanodine receptors (Ca(2+)-release channels). Trends Biochem Sci 22:193–194

Reddy BA, Etkin LD, Freemont PS (1992) A novel zinc finger coiled-coil domain in a family of nuclear proteins. Trends Biochem Sci 17:344–345

Reymond A, Meroni G, Fantozzi A, Merla G, Cairo S, Luzi L, Riganelli D, Zanaria E, Messali S, Cainarca S, Guffanti A, Minucci S, Pelicci PG, Ballabio A (2001) The tripartite motif family identifies cell compartments. EMBO J 20:2140–2151

Rhodes DA, de Bono B, Trowsdale J (2005) Relationship between SPRY and B30.2 protein domains. Evolution of a component of immune defence? Immunology 116:411–417

Sakuma R, Noser JA, Ohmine S, Ikeda Y (2007) Rhesus monkey TRIM5alpha restricts HIV-1 production through rapid degradation of viral Gag polyproteins. Nat Med 13:631–635

Sardiello M, Cairo S, Fontanella B, Ballabio A, Meroni G (2008) Genomic analysis of the TRIM family reveals two groups of genes with distinct evolutionary properties. BMC Evol Biol 8:225

Sawyer SL, Wu LI, Emerman M, Malik HS (2005) Positive selection of primate TRIM5alpha identifies a critical species-specific retroviral restriction domain. Proc Natl Acad Sci USA 102:2832–2837

Sawyer SL, Emerman M, Malik HS (2007) Discordant evolution of the adjacent antiretroviral genes TRIM22 and TRIM5 in mammals. PLoS Pathog 3:e197

Sayah DM, Sokolskaja E, Berthoux L, Luban J (2004) Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. Nature 430:569–573

Schultz DC, Ayyanathan K, Negorev D, Maul GG, Rauscher FJ 3rd (2002) SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. Genes Dev 16:919–932

Sebastian S, Luban J (2005) TRIM5alpha selectively binds a restriction-sensitive retroviral capsid. Retrovirology 2:40

Sebastian S, Grutter C, de Castillia CS, Pertel T, Olivari S, Grutter MG, Luban J (2009) An invariant surface patch on the TRIM5alpha PRYSPRY domain is required for retroviral restriction but dispensable for capsid binding. J Virol 83:3365–3373

Sewram S, Singh R, Kormuth E, Werner L, Mlisana K, Karim SS, Ndung'u T (2009) Human TRIM5alpha expression levels and reduced susceptibility to HIV-1 infection. J Infect Dis 199:1657–1663

Shi M, Deng W, Bi E, Mao K, Ji Y, Lin G, Wu X, Tao Z, Li Z, Cai X, Sun S, Xiang C, Sun B (2008) TRIM30 alpha negatively regulates TLR-mediated NF-kappa B activation by targeting TAB 2 and TAB 3 for degradation. Nat Immunol 9:369–377

Short KM, Cox TC (2006) Subclassification of the RBCC/TRIM superfamily reveals a novel motif necessary for microtubule binding. J Biol Chem 281:8970–8980

Sivaramakrishnan G, Sun Y, Rajmohan R, Lin VC (2009a) B30.2/SPRY domain in tripartite motif-containing 22 is essential for the formation of distinct nuclear bodies. FEBS Lett 583:2093–2099

Sivaramakrishnan G, Sun Y, Tan SK, Lin VC (2009b) Dynamic localization of tripartite motif-containing 22 in nuclear and nucleolar bodies. Exp Cell Res 315:1521–1532

Sokolskaja E, Berthoux L, Luban J (2006) Cyclophilin A and TRIM5alpha independently regulate human immunodeficiency virus type 1 infectivity in human cells. J Virol 80:2855–2862

Song B, Diaz-Griffero F, Park DH, Rogers T, Stremlau M, Sodroski J (2005) TRIM5alpha association with cytoplasmic bodies is not required for antiretroviral activity. Virology 343:201–211

Stremlau M, Owens CM, Perron MJ, Kiessling M, Autissier P, Sodroski J (2004) The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. Nature 427:848–853

Stremlau M, Perron M, Welikala S, Sodroski J (2005) Species-specific variation in the B30.2(SPRY) domain of TRIM5alpha determines the potency of human immunodeficiency virus restriction. J Virol 79:3139–3145

Stremlau M, Perron M, Lee M, Li Y, Song B, Javanbakht H, Diaz-Griffero F, Anderson DJ, Sundquist WI, Sodroski J (2006) Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5alpha restriction factor. Proc Natl Acad Sci USA 103:5514–5519

Tissot C, Mechti N (1995) Molecular cloning of a new interferon-induced factor that represses human immunodeficiency virus type 1 long terminal repeat expression. J Biol Chem 270:14891–14898

Tissot C, Taviaux SA, Diriong S, Mechti N (1996) Localization of Staf50, a member of the Ring finger family, to 11p15 by fluorescence in situ hybridization. Genomics 34:151–153

Towers G, Bock M, Martin S, Takeuchi Y, Stoye JP, Danos O (2000) A conserved mechanism of retrovirus restriction in mammals. Proc Natl Acad Sci USA 97:12295–12299

Turelli P, Doucas V, Craig E, Mangeat B, Klages N, Evans R, Kalpana G, Trono D (2001) Cytoplasmic recruitment of INI1 and PML on incoming HIV preintegration complexes: interference with early steps of viral replication. Mol Cell 7:1245–1254

Uchil PD, Quinlan BD, Chan WT, Luna JM, Mothes W (2008) TRIM E3 ligases interfere with early and late stages of the retroviral life cycle. PLoS Pathog 4:e16

Vichi A, Payne DM, Pacheco-Rodriguez G, Moss J, Vaughan M (2005) E3 ubiquitin ligase activity of the trifunctional ARD1 (ADP-ribosylation factor domain protein 1). Proc Natl Acad Sci USA 102:1945–1950

Virgen CA, Kratovac Z, Bieniasz PD, Hatziioannou T (2008) Independent genesis of chimeric TRIM5-cyclophilin proteins in two primate species. Proc Natl Acad Sci USA 105:3563–3568

Wilson SJ, Webb BL, Ylinen LM, Verschoor E, Heeney JL, Towers GJ (2008) Independent evolution of an antiviral TRIMCyp in rhesus macaques. Proc Natl Acad Sci USA 105:3557–3562

Wolf D, Goff SP (2007) TRIM28 mediates primer binding site-targeted silencing of murine leukemia virus in embryonic cells. Cell 131:46–57

Wolf D, Goff SP (2009) Embryonic stem cells use ZFP809 to silence retroviral DNAs. Nature 458:1201–1204

Wolf D, Hug K, Goff SP (2008) TRIM28 mediates primer binding site-targeted silencing of Lys1, 2 tRNA-utilizing retroviruses in embryonic cells. Proc Natl Acad Sci USA 105:12521–12526

Woo JS, Imm JH, Min CK, Kim KJ, Cha SS, Oh BH (2006a) Structural and functional insights into the B30.2/SPRY domain. EMBO J 25:1353–1363

Woo JS, Suh HY, Park SY, Oh BH (2006b) Structural basis for protein recognition by B30.2/SPRY domains. Mol Cell 24:967–976

Yamauchi K, Wada K, Tanji K, Tanaka M, Kamitani T (2008) Ubiquitination of E3 ubiquitin ligase TRIM5 alpha and its potential role. FEBS J 275:1540–1555

Yap MW, Nisole S, Lynch C, Stoye JP (2004) Trim5alpha protein restricts both HIV-1 and murine leukemia virus. Proc Natl Acad Sci USA 101:10786–10791

Yap MW, Nisole S, Stoye JP (2005) A single amino acid change in the SPRY domain of human Trim5alpha leads to HIV-1 restriction. Curr Biol 15:73–78

Yoshimi R, Chang TH, Wang H, Atsumi T, Morse HC 3rd, Ozato K (2009) Gene disruption study reveals a nonredundant role for TRIM21/Ro52 in NF-kappaB-dependent cytokine expression in fibroblasts. J Immunol 182:7527–7538

Zhang F, Hatziioannou T, Perez-Caballero D, Derse D, Bieniasz PD (2006) Antiretroviral potential of human tripartite motif-5 and related proteins. Virology 353:396–409

Zhang F, Perez-Caballero D, Hatziioannou T, Bieniasz PD (2008) No effect of endogenous TRIM5alpha on HIV-1 production. Nat Med 14:235–236 (author reply 236–238)