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The Cell Biology of TRIM5 α

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Abstract

The tripartite motif (TRIM)-containing proteins are involved in many cellular functions such as cell signaling, apoptosis, cell differentiation, and immune modulation. TRIM5 proteins, including TRIM5 α and TRIM-Cyp, are known to possess antiretroviral activity against many different retroviruses. Besides being retroviral restriction factors, TRIM5 proteins participate in other cellular functions that have recently emerged in the study of TRIM5 α . In this review, we discuss properties of TRIM5 α such as cytoplasmic body formation, protein turnover, and trafficking. Also, we discuss recent insights into innate immune modulation mediated by TRIM5 α , highlighting the various functions TRIM5 α has in cellular processes.

Keywords

rhTRIM5 α ; Cytoplasmic bodies; HIV-1; Restriction factor; Signaling 2

Introduction

TRIM5 α is a cellular protein that binds to determinants present on a retroviral capsid soon after the entry of the viral core into the cytoplasm of a target cell. This restriction occurs in a species-specific manner, the best characterized example being the TRIM5 α protein from rhesus macaques (rhTRIM5 α), which potently inhibits HIV-1 infection [1]. It is becoming increasingly clear that TRIM5 α does not simply bind to the retroviral capsid and mediate its effects autonomously without other associated cellular proteins. Rather, the proteins that associate with TRIM5 α and the signaling pathways it activates are intimately tied to its ability to restrict retroviral infection. This review will focus on our understanding of how the cellular biology of TRIM5 α governs its ability to act as an antiviral restriction factor.

TRIM Family of Proteins

The tripartite motif (TRIM) family of proteins is defined by three domains present in all TRIM family members: RING, BBox2, and coiled-coil (CC) [2]. These motifs can be functionally divided into two activities. Firstly, an E3 ubiquitin ligase activity mediated by the N-terminal RING domain. Secondly, self association activity collectively mediated by the BBox2 and CC domains, and at least in the case of TRIM5 α , residues within the L2 region connecting the CC and C-terminal B30.2/PRYSPRY domain. This tendency to self-associate is a defining feature of TRIM family proteins [2], and likely a key aspect of the biology of these proteins, as discussed in more detail below. The tendency to self-

associate has also made this family of proteins quite difficult to study, as TRIM proteins are generally not very soluble in solution at concentrations needed for many standard biochemical and molecular techniques.

TRIM5 α proteins contain an additional C-terminal B30.2/PRYSPRY domain that binds to capsid and mediates specific recognition and restriction of retroviruses by TRIM5 α proteins from different species [1, 13]. For example, while human TRIM5 α (huTRIM5 α) does not restrict HIV-1, as does its counterpart from rhesus macaques (rhTRIM5 α), a single amino-acid change (P332R) is sufficient to lead to HIV-1 restriction by human TRIM5 α [14]. B30.2/PRYSPRY has been subjected to intense positive selection during primate evolution, underscoring its critical role in this process [15]. Space constraints prevent us from describing in detail the many noteworthy studies which describe how variations within this region define the spectrum of retroviral restriction of individual TRIM5 α proteins by modulating the ability to bind retroviral capsid cores, but we refer interested readers to more extensive reviews of this topic [16–18]. Additionally, in some monkey species, the C-terminal SPRY domain has been functionally replaced by a cyclophilin A (CypA) domain [19–22]. These proteins, termed TRIM-Cyp proteins, retain the ability to bind retroviral capsids through the interaction between CypA and the CypA-binding loop of the viral capsid protein.

TRIM5 Multimerization

As TRIM5 self-association is mediated by numerous regions of the protein, self-association can functionally be reduced to low-order (dimerization) and higher-order multimerization. Dimerization is required for higher-order multimerization of TRIM5 proteins, as evidenced by the fact that mutations that perturb dimerization abrogate TRIM5 higher-order multimerization [7].

Dimerization

Defining the low-order multimerization state of TRIM5 α has proven to be complex. TRIM5 α was originally reported to be a trimer [10], although subsequent studies have determined that this finding was a result of an aberrant electrophoretic mobility of TRIM5 α dimers, which are actually the low-order multimerization state of TRIM5 proteins [8, 9]. To circumvent this and other experimentally confounding aspects of TRIM5 α , a recombinant TRIM5 α variant was constructed, in which the RING domain of TRIM5 α was replaced with the RING domain of TRIM21 (TRIM5-21R) [8, 9]. This protein still effectively restricts HIV-1 and has proven to be much more amenable to biochemical purification and subsequent analysis. The dimeric form of TRIM5-21R can directly bind restriction-sensitive viral capsid, while the monomeric form could not [9], demonstrating the functional requirement of this low-order dimerization in TRIM5 α retroviral restriction.

Higher-Order Oligomerization

TRIM5 proteins also exhibit higher-order oligomerization. Using a protein cross-linking reagent such as glutaraldehyde or EGS, TRIM5 α is crosslinked into numerous high-molecular weight species [7, 10, 12, 23–26]. While the apparent molecular weight of these crosslinked species is often variable, at least one study has found that biochemical crosslinking yields a species with a molecular weight consistent with a hexameric form of TRIM5 α [26]. The BBox2 domain appears to be a critical determinant in mediating the higher-order multimerization. A hydrophobic patch on the surface of the B-box 2 domain is necessary for the higher-order multimerization of TRIM5 α as well as capsid binding and retroviral restriction [4, 23, 25, 27]. These observations suggest an important role for higher-order multimerization in the ability of TRIM5 α to restrict retroviral infection. It is well

accepted that the C-terminal B30.2/PRYSPRY domain (in the case of TRIM5 α) or CypA domain (in the case of TRIM-Cyp proteins) are the regions of the protein that directly bind viral capsid determinants. Therefore, the finding that interfering with the ability of TRIM5 α to form higher-order multimers, disrupts the ability to bind in vitro assembled capsid cores suggests that binding of TRIM5 to these complexes requires cooperativity between individual TRIM5 dimers. In such a model, the binding of the first TRIM5 protein to the viral capsid enhances the ability of subsequent TRIM5 proteins to be recruited to the complex and bind the capsid in a C-terminal domain-dependent fashion.

We have also recently identified residues within the Linker 2, which are necessary for the ability of TRIM5 α to self-associate and restrict HIV-1 infection [18]. However, the higher-order multimerization mediated by the L2 region appears, in some ways, distinct from the higher-order multimerization mediated by the BBox2 region. There are two stretches of amino acid residues in L2 which, if altered, abrogate the ability of TRIM5 α to restrict HIV-1 infection. TRIM5 α variants harboring mutations in these regions also exhibit a diffuse localization in cells and fail to localize to cytoplasmic bodies. However, biochemical crosslinking does not reveal an apparent defect in the ability of these mutants to form higher-order multimers [18].

A recent study by Ganser-Pornillos et al. [28•] has provided valuable insight into the nature of the assemblies formed by TRIM5 α . Using negative-stain EM and biochemical studies, these authors demonstrated that TRIM5-21R spontaneously forms two-dimensional hexagonal arrays. These TRIM5 arrays are complementary in symmetry and dimension to the retroviral capsid. The authors suggest that retroviral capsid binding and higher-order assemblies are coupled. Their model proposes that the B-Box2 domain mediates tripodial protein extensions observed in the lattice, while the L2 region mediates the self-association of opposing dimers needed to achieve hexagonal symmetry [28•].

TRIM5 α Cytoplasmic Bodies: Are all Bodies Created Equal?

The paper which first characterized the TRIM family of proteins observed that most TRIM family members localized to discrete accumulations of proteins of diverse shapes and sizes in the nucleus and the cytoplasm [2]. TRIM5 α localizes to structures termed cytoplasmic bodies [1]. The importance of these cytoplasmic bodies (CBs) to retroviral restriction has been controversial since the first discovery of TRIM5 α 's antiviral activity. Our studies have shown that TRIM5 α cytoplasmic bodies are highly dynamic structures that rapidly exchange between cytoplasmic bodies and a diffuse cytoplasmic fraction [29], suggesting that these accumulations to which TRIM5 α localizes are not simply dead end aggregates. We have also used live cell imaging to observe the de novo formation of rhTRIM5 α cytoplasmic bodies around fluorescently labeled HIV-1 virions [30•]. However, two studies show that preexisting cytoplasmic bodies are not required for TRIM5 α 's antiretroviral activity. One study used geldanamycin, which is an Hsp90 inhibitor, prior to infection in order to reduce or eliminate TRIM5 α cytoplasmic body formation. In this study geldanamycin treatment did not affect the ability of the TRIM5 α proteins to restrict HIV-1 infection despite its ability to disrupt cytoplasmic bodies [31]. Another study utilized a cell line expressing epitope tagged TRIM-Cyp which does not form cytoplasmic bodies in the absence of restriction-sensitive virus and found that this cell line was able to efficiently restrict HIV-1 infection [32]. While these studies did not determine if CBs formed following encounter with restriction-sensitive virus, they collectively demonstrate that the preexisting pool of CBs is not required for retroviral restriction.

The disagreement regarding the role that CBs play in restriction is complicated both by the inability to assess the localization of endogenous TRIM5 proteins and also a lack of

consensus regarding the definition of what constitutes a cytoplasmic body. Because reliable antibodies do not exist that can with certainty assess the localization of TRIM5 α proteins in cells, most studies assessing its localization have relied on the expression of epitope-tagged forms of the protein. This raises the concern that the localization observed in epitope-tagged cell lines or following transient transfection may be an artifact of protein overexpression and may not resemble the localization of endogenous protein. The formation of these structures is certainly dependent on expression levels, as there are striking differences in the localization of epitope-tagged TRIM5 α proteins in transiently transfected cells or cells expressing TRIM5 α from a stably integrated retroviral vector (Fig. 1). Transient transfection yields very large and protein dense TRIM5 α accumulations in cells, while a more diffuse localization with fewer, smaller cytoplasmic puncta are commonly observed in stably expressing cell lines. Notably, transiently transfected cells inhibit retroviral infection poorly if at all, while stably expressing cells, which express TRIM5 α at much lower levels, inhibit retroviral infection much more potently (EMC, unpublished observations). This suggests that the localization of TRIM5 α following transient transfection is aberrant in a way that disrupts the antiviral function of TRIM5 α .

If the large aggregates formed following transient transfection are not biologically relevant structures, what about the smaller structures visible in stable cell lines? It is clear that the interaction between TRIM5 α and viral capsids in the cytoplasm of target cells is relevant to TRIM5 α restriction, so it seems very likely that the TRIM5 α assemblies observed to form around HIV-1 capsids, visible by live and fixed cell imaging [30•], are relevant to the ability of TRIM5 α to restrict retroviral infection. However, aside from the fact that they can be observed associating with viral capsids, these cytoplasmic concentrations of TRIM5 α are indistinguishable from the puncta that exist in the absence of virus by fluorescent microscopy. It is possible that TRIM5 α accumulations visible in the absence of virus represent protein assemblies that are fundamentally similar to those that contain a restricted retrovirus. The study of the hexameric lattices formed by TRIM5-21R support this notion [28•]. This study found that TRIM5-21R formed identical lattices in the presence or absence of restriction-sensitive virus, though the formation was enhanced by the addition of in vitro assembled viral capsid structures [28•]. It therefore seems possible that the puncta visible in cells in the absence of virus represent the in situ manifestation of this observation. Moving forward, it will be valuable to understand the differences and similarities between these microscopically similar cytoplasmic accumulations of TRIM5 α , specifically with respect to the other proteins present in both structures. It may be that accumulations of protein which do not contain viral capsids represent small model systems that resemble the assemblies of TRIM5 α that form around viral capsids. It may also be that these accumulations that exist in the absence of virus are more relevant to the capsid-independent signaling aspect of TRIM5 α [33•, 34], described in more detail below. It is also not currently possible to exclude the possibility that these TRIM5 α accumulations that exist in the absence of virus are indeed biologically irrelevant aggregates. Given the observations by Ganser-Pornillos et al. [28•], we favor the idea that these structures are similar in nature to the assemblies which form around restriction-sensitive virus.

When pondering the biological relevance of TRIM5 α CBs, it may be relevant to consider the case of TRIM19, also known as promyelocytic leukemia (PML) protein. Unlike other TRIM family members, including TRIM5 α , a number of antibodies exist which are commonly used to detect endogenously expressed PML by immunofluorescence [35–37]. These antibodies can be used to detect PML localizing to nuclear structures termed nuclear bodies (NBs), which are electrondense spheres of 0.1–1.0 μ M in diameter [38]. Unlike the situation with TRIM5 α , the relevance of these structures formed by PML is not a matter of debate. While the extensive literature on PML bodies is outside of the scope of this review, we refer the interested reader to numerous review articles describing the role of these bodies

and these structures in cancer, antiviral defense, and their dynamic response to cellular stress and viral infection [38–40]. Notably, the study of PML NBs not only reveals many similarities between PML NBs and TRIM5 α cytoplasmic bodies, they may also associate with each other in some cases, as discussed in more detail below.

Intracellular TRIM5 α Trafficking

Studies of the intracellular mobility of TRIM5 α proteins have revealed a dynamic protein which exhibits mobility at many levels. Fluorescence recovery after photobleaching (FRAP) and photo-activatable GFP (PA-GFP) studies have demonstrated that there is a rapid exchange of TRIM5 α which occurs between proteins present in CBs and the diffuse cytoplasmic pool of TRIM5 α [29]. Notably, the rate of TRIM5 α exchange in these structures, is very comparable to the rate of exchange measured for PML NBs in similar studies [41, 42]. As is also the case with PML NBs [43, 44], TRIM5 α CBs can be observed to exhibit dynamic fission and fusion behaviors, allowing the formation of multiple CBs from larger precursors or the coalescence of individual CBs into larger CBs [29]. However, the relevance of these dynamic behaviors is not well understood. We have observed that individual TRIM5 α CBs can dynamically transfer TRIM5 α onto HIV-1 virions during restriction [30•], and the relevance of this transfer must be considered in the context of the previously mentioned studies demonstrating that preexisting CBs are not required for retroviral restriction [31, 32]. It is also noteworthy that the movement of TRIM5 α CBs is not random, possibly limiting it to regions where the incoming retroviral core is located.

TRIM5 α CBs are transported along microtubules [29]. Live cell imaging experiments readily show TRIM5 α CBs rapidly trafficking along curvilinear paths, and this trafficking is sensitive to the microtubule destabilizing agent nocodazole. The mechanism by which TRIM5 α CBs engage the microtubule trafficking machinery is unclear. While other TRIM family proteins have been shown to associate with microtubules [45], the proposed mechanism of this interaction involves a C-terminal COS box domain not present in TRIM5 α proteins [45]. Recent studies seem to suggest that the association between TRIM5 α and microtubules may be relevant to restriction, as disruption of microtubules by nocodazole treatment reduces restriction of HIV-1 mediated by TRIM5 α (Paulina Pawlica and Lionel Berthou, personal communication). Incoming HIV-1 viral complexes have also been reported to be transported by the microtubule network during infection [46]. Association with microtubules may provide an opportunity for TRIM5 α to effectively decrease the required area of retroviral surveillance, preferring to hunt for susceptible retroviral cores along the two-dimensional microtubule network rather than the vastly larger three-dimensional cytoplasm.

It was recently reported that some TRIM5 α proteins traffic through the nucleus, even though their steady state localization is primarily cytoplasmic (Fig. 1). This study utilized leptomycin B (LMB), an inhibitor of CRM1-mediated nuclear export, to show that rhTRIM5 α and huTRIM5 α , but not owl monkey TRIM-Cyp and other primates, can accumulate in the nucleus when CRM1 nuclear export is inhibited [47]. Notably, nuclear accumulation of these proteins results in their localization to PML NBs. While the relevance of this nuclear trafficking is not well understood, it may be related to a recent study showing that SUMO interacting motifs (SIMs) present in TRIM5 α are required for its ability to restrict HIV-1 infection [48]. SUMO-1 localization is predominantly nuclear (EMC, unpublished observations) and PML sumoylation is known to be important for the formation of PML NBs [39].

TRIM5 Turnover

While there appears to be a connection between TRIM5 α turnover and its ability to restrict infection, this connection has proven to be less than straightforward. Pulsechase analysis and cycloheximide studies demonstrate the approximate half-life of TRIM5 α to be slightly less than an hour [23, 49]. This rate of turnover is only slightly increased by the addition of proteasome inhibitors [23, 49], suggesting that a proteasome-independent pathway mediates the rapid turnover of TRIM5 α in absence of restriction-sensitive virus. In two different studies, the ability of rhTRIM5 α to restrict HIV-1 does not strictly correlate with the half-life of the protein in the absence of virus [18, 23].

Yet, it is known that inhibition of the proteasome by MG132 relieves the TRIM5 α -mediated block of reverse transcription products while not affecting the ability to inhibit infection [49, 50]. Another study has demonstrated that, following addition of restriction-sensitive virus, TRIM5 α is degraded in a proteasome-dependent fashion [51]. This data would suggest that the degradative fate of TRIM5 α is altered in presence of virus. While it is unclear how this switch is mediated, we have recently reported that TRIM5 α associates with the protein p62/sequestosome-1 [52], which is an adaptor protein that has been reported to facilitate both the proteasomal [53–55] and autophagic [56] degradation of cellular proteins. p62 somehow acts to stabilize TRIM5 α expression, as p62 depletion by siRNA increases the rate of TRIM5 α turnover in cells in the absence of virus [52].

Innate Immune Signaling and E3 Ubiquitination Activity

Various TRIMs have been shown to play a part in host immunity and signaling pathways [57–64]. However until recently, it was not appreciated that TRIM5 proteins are capable of stimulating intracellular pathways. One study exploring this possibility observed that both human and rhesus TRIM5 α are able to activate NF- κ B-driven transcription in the absence of restriction-sensitive virus [34]. Another study from Pertel et al. [33•] similarly observed the ability of TRIM5 proteins to activate NF- κ B and AP-1 transcription in a capsid-independent fashion. Both studies also observe that TRIM5 proteins can bind the signaling adapter proteins TAB2 and TAB3, though this binding was not strictly correlated with the ability to activate NF- κ B, suggesting that the ability of TRIM5 proteins to activate this pathway may be multifaceted [33•, 34]. Notably, the study by Pertel and colleagues also observed that the basal signaling activity of TRIM5 proteins is stimulated by the addition of restriction-sensitive virus and that association with components of this signaling cascade, most notably TAK1, is required for efficient retroviral restriction [33•]. These authors found that TRIM5 synthesizes unanchored K63-linked ubiquitin chains which activate TAK1 autophosphorylation, further stimulating AP-1 and NF- κ B signaling.

The observations that TRIM5 associates with TAK1 and synthesizes unanchored K63-linked ubiquitin raise a number of critical questions about TRIM5 proteins and restriction. It is known that TRIM5 recognizes incoming viral capsid within minutes of their entry into the cytoplasm. In the case of TRIM-Cyp, this recognition is irreversible with subsequent treatment of cells with cyclosporin A (CsA) [32]. On the surface, this seems incompatible with the idea that TRIM5 association with signaling molecules induces the de novo expression of a cofactor critical for restriction. Perhaps TRIM5 proteins sequester restriction-sensitive virions for some period while the products of the signaling cascades they activate are induced. If this sequestered complex was somehow resistant to CsA neutralization, this could explain the apparent discordance between these two studies. It is also possible that TAK1 has a more direct role in the poorly defined events that mediate restriction. For example, perhaps TAK1 phosphorylates a viral or cellular protein directly in a manner that is required for subsequent events in restriction, independent of its role in

signal transduction. Further studies will clarify the interesting role TAK1 and related signaling proteins play in TRIM5-mediated restriction.

The study by Pertel et al. also demonstrates that the relationship between restriction and TRIM5 E3 ligase activity is more complex than previously appreciated. Examination of TRIM5 activity in vitro has demonstrated that TRIM5 can auto-ubiquitinate itself in the presence of the E2 enzyme UbcH5B [65]. A subsequent study demonstrated a similar capacity for TRIM5 auto-ubiquitination in the presence of UbcH5a [66]. However, the formation of unanchored K63-linked ubiquitin chains in the Pertel study required the E2 enzyme UBC13-UEV1A. Knockdown of UBC13 rescued HIV-1 and EIAV from TRIM5-mediated restriction. These results are not necessarily discordant, as both types of TRIM5 E3 ligase activity may be relevant to retroviral restriction, other aspects of the cell biology of TRIM5 proteins, or perhaps both. It seems feasible that TRIM5 ligase activity may be regulated in a spatiotemporal fashion. Perhaps encounter with a restriction-sensitive retrovirus facilitates the formation of an assembly that favors association with one particular E2 enzyme over another, thus altering the nature of TRIM5 E3 ligase activity exhibited by TRIM5 associated with a retroviral core as compared to the activity it exhibits when in the diffuse cytoplasmic pool of TRIM5 or in preexisting CBs that exist in the absence of viral capsid. Future studies will surely aim to test these possibilities, providing a more clear picture of how TRIM5 participation in signal transduction cascades and alterations in TRIM5 E3 ligase activity relate to the ability of TRIM5 proteins to restrict retroviral infection.

Conclusions

TRIM5 proteins are intrinsic restriction factors which bind to determinants present in retroviral capsids and prevent infection in a species-specific manner. However, it now seems clear that the antiviral activity of these proteins depends on dynamic interactions with additional cellular proteins. A better understanding of the cell biology of TRIM5 α and related cellular factors will likely continue to provide critical insights into the restriction mechanism mediated by TRIM5 proteins.

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Fig. 1.

Subcellular localization of rhesus macaque TRIM5α. A, HeLa cells were seeded on glass coverslips and transiently transfected with YFP-rhTRIM5α using a PEI protocol. Z-stack images were collected and de-convolved using Deltavision deconvolution software. Individual channel images were superimposed to create the merged image. B, HeLa cells stably expressing YFP-rhTRIM5α from an integrated retroviral vector were seeded on coverslips. Z-stack images were collected and de-convolved using Deltavision deconvolution software. Individual channel images were superimposed to create the merged image