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Human Trim5α has additional activities that are uncoupled from retroviral capsid recognition

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ABSTRACT

Trim5α is a host antiviral protein that recognizes incoming retroviral capsids in the cytoplasm and prevents productive infections. Although present in most mammals, the state of the *Trim5* gene is dynamic in that primates have one copy with several splice variants, while rodents and cows have multiple copies. Mouse Trim30 (one of the mouse Trim5α homologs) has been shown to negatively regulate NF-kappaB activation by targeting upstream signaling intermediates TAB2 and TAB3 for degradation. We show that human Trim5α also affects levels of TAB2, resulting in abrogation of TAB2-dependent NF-kappaB activation. Surprisingly, unlike mouse Trim30, human and rhesus Trim5α are able to activate NF-kappaB-driven reporter gene expression in a dose-dependent manner. We show that Trim5α uses distinct domains for the distinct abilities of affecting TAB2 levels, regulating NF-kappaB, and recognizing retroviral capsids. Our results demonstrate functions of Trim5α that are not dependent on recognizing the retroviral capsid.

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Introduction

Trim5α is a host antiviral protein initially identified as a factor in rhesus macaque monkeys that blocks human immunodeficiency virus-1 (HIV-1) infection during an early post-entry stage (Stremlau et al., 2004) (reviewed in Towers, 2005). Proteins such as Trim5α that are part of the interface between host and pathogen undergo adaptive evolution which manifests itself in the form of a higher rate of nonsynonymous changes compared to synonymous changes (Holmes, 2004). Analysis of rapidly evolving codons among primate Trim5α orthologs has identified amino acids that determine the specificity of Trim5α towards retroviral capsids (Sawyer et al., 2005). These amino acids are mostly located in the PRY-SPRY domain due to interactions between this domain and retroviral capsids (Sawyer et al., 2005; Liu et al., 2005; Stremlau et al., 2004; Sebastian and Luban, 2005).

While the PRY-SPRY domain has been rapidly evolving, the other domains of the protein including the RING, Bbox, and coiled-coil (RBCC) domains (Nisole et al., 2005; Reymond et al., 2001) show more sequence conservation (Sawyer et al., 2005, 2007; Tareen et al., 2009; Johnson and Sawyer, 2009). The reasons for the evolutionary constraint in these more conserved domains could be due to maintaining structural integrity, catalytic functions, and/or other unknown functions. For example, the RING and Bbox domains of Trim5α perform effector functions and contain characteristic zinc-finger motifs (Stremlau et al., 2004; Perez-Caballero et al., 2005), while the coiled-coil domain allows multimerization (Mische et al., 2005). Although some of the constraint in the RBCC domains of Trim5α could be explained by the need to maintain functional zinc-finger motifs in the RING and Bbox and by the need to maintain the ability to multimerize through the coiled coil domain, the question still remains whether Trim5α harbors other unknown functions in addition to its role as an antiviral restriction factor.

The *Trim5* locus has undergone expansions on more than one occasion, such that in cows and rodents, there have been two independent paralogous expansions of *Trim5* genes (reviewed in Johnson and Sawyer, 2009). Cows have up to five *Trim5* genes (Sawyer et al., 2007; Si et al., 2006), while rats have three and mice have up to eight (Tareen et al., 2009). Two of the mouse *Trim5* genes were previously known as *Trim12* and *Trim30*. However, phylogenetic analysis of these two genes and their paralogs (named *Trim12-1*, *12-2*, and *Trim30-1*, *30-2*, *30-3*, and *30-4*) revealed that they all are, in fact, homologs of *Trim5* (Tareen et al., 2009). One of these mouse *Trim5* genes, known as *Trim30*, has been shown to negatively regulate Toll-like receptor (TLR)-mediated nuclear factor-kappaB (NF-kappaB) activation by targeting TAB2 and TAB3 for degradation (Shi et al., 2008). Although at the time, it was not appreciated that mouse Trim30 was a homolog of

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primate Trim5α (Bowie, 2008; Shi et al., 2008), this result suggested that Trim5α might have additional roles in innate immunity besides direct recognition of retroviral capsids.

TAB2 and TAB3 are two adaptor proteins that play a key role in activation of a kinase called TAK1 (TGF-beta activated kinase 1) (Cheung et al., 2004; Takaesu et al., 2000; Yamaguchi et al., 1995), resulting in downstream activation of members of the mitogen activated protein kinase (MAPK) family that are indispensable for several signaling pathways such as NF-kappaB, IL-1beta, TNF, and TLR signaling (reviewed in Chen et al., 2006; Delaney and Mlodzik, 2006). Upstream ligand–receptor interactions are transmitted via TAK1 to downstream MAPKs as well as to NF-kappaB through phosphorylation of its inhibitor IkB kinase (IKK) (Chen, 2005; Shim et al., 2005). Expression of mouse Trim30 targets both TAB2 and TAB3, thus abrogates the activation of TAK1 and the subsequent downstream activation of NF-kappaB (Shi et al., 2008). These observations raise the question of whether this function is conserved across Trim5α homologs from all mammals.

Here, we find that the ability to affect levels of TAB2 is a conserved function of Trim5α in mice and humans. Use of proteasomal inhibitors as well as RING domain zinc finger mutants of Trim5α demonstrate that Trim5α affects TAB2 levels through a mechanism that is independent of E3 ubiquitin ligase activity. The ability to affect TAB2 levels is present in human and mouse Trim5α, but not in rhesus Trim5α. Like mouse Trim5α, human Trim5α also abrogates TAB2-dependent NF-kappaB activation. However, unlike mouse Trim5α, both human and rhesus Trim5α are able to independently activate NF-kappaB-driven transcription. By defining the domains of human Trim5α necessary for these functions, we were able to genetically separate the ability of Trim5α to affect TAB2 levels from its ability to activate NF-kappaB. These independent abilities of Trim5α did not require the adaptively evolving retroviral capsid recognizing PRY-SPRY domain. Our results demonstrate functions of Trim5α that are not dependent on recognizing the retroviral capsid and may point to an independent role of Trim5α in the innate immune response to pathogens.

Results

A conserved function for murine Trim5α paralogs and human Trim5α: Affecting levels of TAB2

The mouse *Trim5* locus consists of eight *Trim5* genes, five of which are predicted to encode the full-length splice variant homologous to Trim5α in humans. These full-length genes are known as *Trim12-1*, *Trim12-2*, *Trim30*, *Trim30-2*, and *Trim30-3* (Tareen et al., 2009). Of these, we have been able to clone and express three from mouse RNA, namely Trim12-2, Trim30, and Trim30-3. Since Trim30 has been shown to target TAB2 and TAB3 for degradation (Shi et al., 2008), we asked if this function is conserved across other mouse Trim5α paralogs.

We cotransfected 293T cells with constant amounts of TAB2 alone or in the presence of increasing amounts of the *TRIM* gene being tested (Fig. 1). Consistent with what has previously been shown (Shi et al., 2008), we found that the levels of murine TAB2 decrease with increasing amounts of Trim30. Trim12-2 and Trim30-3 behaved the same way such that increasing amounts of Trim12-2 or Trim30-3 resulted in a decrease in the levels of TAB2 (Fig. 1). This concentration-dependent manner in which TAB2 levels are affected is specific to mouse Trim5α paralogs since Trim34-1, the next closest related gene that sits near the *Trim5* locus, but is not one of the *Trim5*α genes (Tareen et al., 2009), was not able to affect the levels of TAB2 (Fig. 1, right side). These results show that affecting the levels of TAB2 is a conserved function of mouse Trim5α paralogs.

Since mouse Trim5α paralogs are able to affect protein levels of TAB2, we asked if this activity is also present in human and rhesus Trim5α proteins. Cotransfecting 293T cells with human TAB2 in the presence of increasing amounts of human Trim5α or empty vector (LPCX) showed that increasing amounts of human Trim5α results in lower levels of human TAB2 (Fig. 2A). However, a closely related TRIM protein in humans, Trim22, was not able to affect TAB2 levels, even in exceedingly large amounts of Trim22 being expressed (Fig. 2A). Human Trim5α was also able to affect the levels of mouse TAB2 (Fig. 2A, right side) and rhesus TAB2 (data not shown), even though rhesus Trim5α was unable to affect the levels of either rhesus or human TAB2 (Fig. 2A). These results show that targeting TAB2 is present among human and murine Trim5α homologs but may not be a universal property of Trim5 since we did not observe it for the rhesus homolog.

To understand the mechanism of how human Trim5α affects TAB2 levels, we asked if a proteasomal pathway requiring E3 ubiquitin ligase activity of Trim5α was involved. We generated a Trim5α protein that contains a mutated zinc finger in its RING domain by substituting cysteine at position 35 to alanine (Trim5α-C35A). Trim5α-C35A was still able to affect TAB2 levels (Fig. 2B) indicating that an intact zinc finger within the RING domain of Trim5α is not required for affecting TAB2, thus making it unlikely that the effect is through an E3 ubiquitin ligase activity of Trim5. We further assessed this by treating cells with either proteasomal inhibitors or lysosomal inhibitors. We found that Trim5α still is able to affect TAB2 levels in the presence of proteasome inhibitors (Fig. 2C). We observed a very slight effect of lysosomal inhibitors compared to no drug control is consistent with mouse Trim30 which also targets mouse TAB2

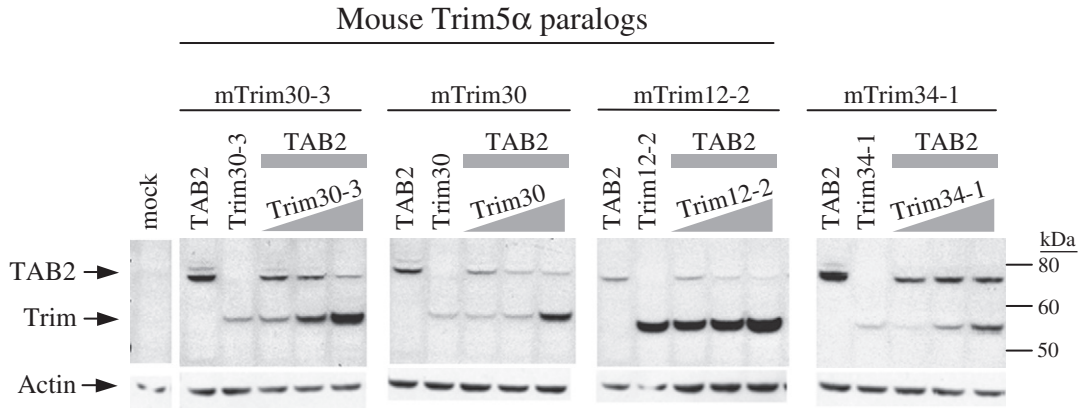

Fig. 1. Targeting TAB2 for degradation is a conserved among mouse Trim5α paralogs. 293T cells were cotransfected with constant amounts of mouse TAB2 (indicated by gray rectangle) and with increasing amounts of either of the mouse Trim5α paralogs (Trim12-2, Trim30, or Trim30-3) or of mouse Trim34-1 (indicated by gray triangle). Total amount of DNA transfected was normalized using empty vector DNA. All constructs contain HA tags. Lysates were probed with anti-HA antibodies, then stripped and reprobed with anti-actin. Mouse Trim is abbreviated as mTrim.

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