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ScienceDirect
Virology 353 (2006) 234–246
VIROLOGY
www.elsevier.com/locate/yviro

Characterization of TRIM5α trimerization and its contribution to human immunodeficiency virus capsid binding

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Received 12 April 2006; returned to author for revision 28 April 2006; accepted 9 May 2006
Available online 30 June 2006

Abstract

The coiled-coil domain of the tripartite motif (TRIM) family protein TRIM5α is required for trimerization and function as an antiretroviral restriction factor. Unlike the coiled-coil regions of other related TRIM proteins, the coiled coil of TRIM5α is not sufficient for multimerization. The linker region between the coiled-coil and B30.2 domains is necessary for efficient TRIM5α trimerization. Most of the hydrophilic residues predicted to be located on the surface-exposed face of the coiled coil can be altered without compromising TRIM5α antiviral activity against human immunodeficiency virus (HIV-1). However, changes that disrupt TRIM5α trimerization proportionately affect the ability of TRIM5α to bind HIV-1 capsid complexes. Therefore, TRIM5α trimerization makes a major contribution to its avidity for the retroviral capsid, and to the ability to restrict virus infection.

Keywords: TRIM5α; HIV-1; Retroviral capsid; Coiled-coil domain; Trimerization

Introduction

The retroviral restriction factor TRIM5α is a member of a family of proteins that contain a tripartite motif, hence the designation TRIM (Reymond et al., 2001). The tripartite motif includes a RING domain, B-box 2 domain, and coiled-coil (cc) domain; TRIM proteins have also been called RBCC proteins. TRIM proteins exhibit the propensity to form cytoplasmic or nuclear bodies (Reymond et al., 2001). Many cytoplasmic TRIM proteins contain a C-terminal B30.2 or SPRY domain. Differential splicing of the *TRIM* primary transcripts gives rise to the expression of several isoforms of the protein products. TRIM5α is the largest TRIM5 isoform (~493 amino acid residues) and contains the B30.2(SPRY) domain.

The TRIM5α proteins of different primate lineages mediate early blocks to infection by particular retroviruses. For example, rhesus monkey TRIM5α (TRIM5α_{rh}) potently inhibits human immunodeficiency virus (HIV-1), whereas human TRIM5α (TRIM5α_{hu}) only modestly blocks HIV-1 infection but efficiently restricts N-tropic murine leukemia virus (N-MLV) infection (Hatziioannou et al., 2004; Keckesova et al., 2004; Perron et al., 2004; Yap et al., 2004; Stremlau et al., 2004). The B30.2(SPRY) domain of rhesus monkey TRIM5α is essential for anti-HIV-1 activity (Stremlau et al., 2004). Several lines of evidence hint that TRIM5α associates directly or indirectly with the restricted retroviral capsid. First, the viral determinants of susceptibility to restriction map to the capsid protein (Stremlau et al., 2004). Second, when introduced into cells, assembled and proteolytically processed capsids of virus-like particles, but not individual capsid proteins, can compete for the restriction factor(s) (Bieniasz, 2003; Goff, 2004; Stoye, 2002). Recently, the B30.2(SPRY) domains have been shown to be essential for TRIM5α_{hu} and TRIM5α_{rh} association with N-MLV and HIV-1 capsid complexes, respectively (Sebastian and Luban, 2005; Stremlau et al., 2006).

Coiled-coil domains have been implicated in the ability of TRIM proteins to form homo-oligomers (Reymond et al., 2001). Coiled-coil domains from various proteins typically exhibit a seven-residue repeat (abcdefg), where a and d

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doi:10.1016/j.virol.2006.05.017

represent hydrophobic amino acids that are responsible for homo-oligomerization (Harbury et al., 1993, 1995; Lumb and Kim, 1995; Lupas et al., 1991). The other residues, which are exposed on the assembled coiled coil, tend to be polar residues. Sequence variation among coiled coils determines oligomerization states and specific functions. Structural predictions suggest that the coiled coils of TRIM proteins exhibit a propensity to form both dimers and trimers (Harbury et al., 1993, 1995; Lupas et al., 1991). Oligomerization has been shown to be important for the function of the nuclear TRIM28 (KAP-1) protein (Peng et al., 2000, 2002). The coiled-coil domain of TRIM5 was shown to be necessary for multimerization (Javanbakht et al., 2005; Perez-Caballero et al., 2005). Recently, TRIM5α proteins from different species have been shown to form trimers (Mische et al., 2005). Deletion of the TRIM5α_{rh} coiled coil disrupted the interaction with HIV-1 CA–NC complexes (Stremlau et al., 2006). Trimerization may allow TRIM5α to interact with sites on the surface lattice of the retroviral capsid that exhibit trimeric pseudosymmetry (Mische et al., 2005). Significant gains in avidity would accrue to the interactions of two oligomeric complexes with compatible symmetry.

Here, we study the requirements for TRIM5α trimerization and define sequences flanking the coiled-coil region that contribute to oligomerization. We study the impact of TRIM5α oligomerization on association with the viral capsid protein and the ability to mediate the restriction of retroviral infection.

Results

Contribution of the TRIM5α_{rh} coiled-coil domain to trimerization and function

Secondary structure predictions (Lupas et al., 1991) indicate that the TRIM5α coiled-coil (CC) domain consists of two regions (CCA and CCB), each of which exhibits a strong propensity to form a coiled coil, separated by a spacer region (Fig. 1A). To investigate the contribution of the TRIM5α_{rh} CC domain to oligomerization and function, we generated several mutants with deletions affecting this domain (Fig. 1A). The wild-type and mutant TRIM5α_{rh} proteins have an influenza hemagglutinin (HA) epitope tag at their carboxyl termini. In the TRIM5α_{rh} ΔCC mutant, the entire CC domain is deleted. In the TRIM5α_{rh} ΔCC GCN4, the deleted TRIM5α_{rh} CC domain is replaced by a heterologous trimeric coiled coil derived by modification of the GCN4 transcription factor (Harbury et al., 1993; Lumb and Kim, 1995). This GCN4 trimeric CC domain has been shown to assist or promote the trimerization of other proteins (Weissenhorn et al., 1997; Yang et al., 2002, 2005). In the TRIM5α_{rh} ΔCCA and ΔCCB mutants, the CCA and CCB predicted coiled coils are respectively deleted. The TRIM5α_{rh} CC NT and CC CT mutants contain each half of the TRIM5α_{rh} protein, with the CCA region in the CC NT mutant and the CCB region in the CC CT mutant.

To examine the expression and oligomerization state of the mutant TRIM5α_{rh} proteins, cross-linking with increasing concentrations of ethylene glycol-bis(succinimidyl succinate) (EGS) was employed. The wild-type TRIM5α_{rh} protein was cross-linked into a 160 kDa species, consistent with a trimer (Fig. 1B). The TRIM5α_{rh} ΔCC mutant, by contrast, did not exhibit higher-order forms upon cross-linking. The trimeric GCN4 motif in the TRIM5α_{rh} ΔCC GCN4 mutant restored the ability of the protein to oligomerize into trimers, with only slightly decreased efficiency compared to the wild-type TRIM5α_{rh} protein. Deletion of the CCA region dramatically reduced the efficiency of trimer formation, although higher-order forms were apparent after cross-linking. The oligomerization of the TRIM5α_{rh} ΔCCB mutant was severely compromised, compared with that of the wild-type TRIM5α_{rh} protein. Neither of the TRIM5α_{rh} CC NT or CC CT mutants exhibited evidence of oligomerization in cross-linking experiments (Fig. 1B). In coimmunoprecipitation experiments, neither of these mutants demonstrated the ability to associate with each other or with the wild-type TRIM5α_{rh} protein (Fig. 1C). We conclude that both the CCA and CCB elements of the TRIM5α_{rh} CC domain are critical for efficient trimerization.

The effect of changes in the TRIM5α_{rh} CC and flanking regions on the subcellular localization of the protein was examined by staining Cf2Th cells stably expressing these variants with an antibody directed against the HA epitope tag (Fig. 1D). The wild-type TRIM5α_{rh} protein was diffusely located throughout the cytoplasm and also concentrated in cytoplasmic bodies, as previously described (Javanbakht et al., 2005; Perez-Caballero et al., 2005). The TRIM5α_{rh} mutants examined, which have deletions of the CC or adjacent linker 2 domain, exhibited diffuse cytoplasmic staining, but did not localize to cytoplasmic bodies (Fig. 1D). We conclude that the TRIM5α_{rh} CC and linker 2 domains are not required for cytoplasmic localization, but may influence TRIM5α_{rh} incorporation into cytoplasmic bodies, perhaps through a contribution to multimerization.

The ability of some of the TRIM5α_{rh} variants to inhibit HIV-1 infection was studied. Cf2Th cells expressing these variants or transduced with the empty LPCX vector were challenged with VSV G glycoprotein-pseudotyped recombinant HIV-1 expressing green fluorescent protein (GFP), and GFP-positive cells were scored (Fig. 1E). Cells expressing wild-type TRIM5α_{rh} protein potently resisted HIV-1 infection, whereas cells expressing the other TRIM5α_{rh} variants tested were infected comparably to the control cells transduced with the empty LPCX vector. Thus, the CC domain is apparently important for the antiviral function of TRIM5α_{rh}.

Definition of the minimum TRIM5α regions required for trimerization

To examine the minimum sequences required for trimerization of TRIM5α and other TRIM proteins, the CC domains of a variety of TRIM family proteins were expressed independently of the rest of the protein. As a control, the GCN4 trimerization domain was expressed. This GCN4 protein and the coiled-coil domains of human TRIM6, TRIM21, TRIM27, and TRIM1 all exhibited the ability to form oligomers (Fig. 2). The TRIM6 and TRIM21 coiled coils formed mostly dimers, whereas dimeric