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Dynamic conformational changes in the rhesus TRIM5α dimer dictate the potency of HIV-1 restriction

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ABSTRACT

The TRIM5α protein from rhesus macaques (rhTRIM5α) mediates a potent inhibition of HIV-1 infection via a mechanism that involves the abortive disassembly of the viral core. We have demonstrated that alpha-helical elements within the Linker 2 (L2) region, which lies between the SPRY domain and the Coiled-Coil domain, influence the potency of restriction. Here, we utilize single-molecule FRET analysis to reveal that the L2 region of the TRIM5α dimer undergoes dynamic conformational changes, which results in the displacement of L2 regions by 25 angstroms relative to each other. Analysis of restriction enhancing or abrogating mutations in the L2 region reveal that restriction defective mutants are unable to undergo dynamic conformational changes and do not assume compact, alpha-helical conformations in the L2 region. These data suggest a model in which conformational changes in the L2 region mediate displacement of CA bound SPRY domains to induce the destabilization of assembled capsid during restriction.

1. Introduction

TRIM5α is a retroviral restriction factor which mediates a post-entry block to infection (Sastri and Campbell, 2011; Stremlau et al., 2004). The most well studied example of this restriction is the ability of the TRIM5α protein from rhesus macaques (rhTRIM5α) to potently inhibit HIV-1 infection (Sastri and Campbell, 2011; Stremlau et al., 2004). Like other members of the TRIM family of proteins, TRIM5α possesses the canonical RING, BBox2, and coiled coil (CC) domains that comprise the TRIpartite Motif that defines this family of proteins (Ozato et al., 2008). Like other TRIM family proteins, TRIM5α exhibits a strong tendency to self-associate into macromolecular assemblies in cells (Cai et al., 2008; Campbell et al., 2007). The N-terminal RING domain of TRIM5α is known to act as an E3 ubiquitin ligase (Pertel et al., 2011; Tareen and Emerman, 2011; Yamauchi et al., 2008; Yudina et al., 2015), and, together with the BBox2 domain, also functions to mediate the self-association of TRIM5α dimers (Diaz-Griffero et al., 2009; Li et al., 2011). The CC domain, in cooperation with the Linker 2 (L2) region, mediates the dimerization of TRIM5α monomers and the formation of higher order assemblies (Goldstone et al., 2014; Kar et al., 2011; Langelier et al., 2008; Sanchez et al., 2014; Sastri et al., 2010). TRIM5α also possesses a C-terminal SPRY domain, which is known to recognize determinants in the assembled viral core to mediate restriction (Ohkura et al., 2006; Stremlau et al., 2005; Yap et al., 2005). Following core binding, TRIM5α induces the abortive disassembly of the viral core (Stremlau et al., 2006; Zhao et al., 2011), although the mechanism by which this abortive disassembly is induced by TRIM5α remains poorly understood.

Structural studies have been valuable in understanding the molecular basis for the interactions between TRIM5α and the HIV-1 capsid (CA) core. Cryo-EM studies have revealed that TRIM5α can form hexagonal assemblies on artificially assembled CA lattices (Ganser-Pornillos et al., 2011; Li et al., 2016). The domain organization of TRIM5α within this assembly has recently been revealed by structural studies revealing that TRIM5α and TRIM25, a closely related TRIM family member, form relatively long antiparallel dimers (Goldstone et al., 2014; Sanchez et al., 2014) (Fig. 1B), the dimensions of which are consistent with these dimeric units spanning each face of the hexagonal lattice observed by cryo-EM (Ganser-Pornillos et al., 2011) (Fig. 1C). As such, the antiparallel dimer consisting of the CC-L2-SPRY domain are thought to represent the basic CA binding unit of TRIM5α, and recombinant proteins comprised of the CC-L2-SPRY domains have been observed to bind assembled CA in vitro (Zhao et al., 2011). Moreover, this minimal CA binding unit, lacking the N-terminal RING

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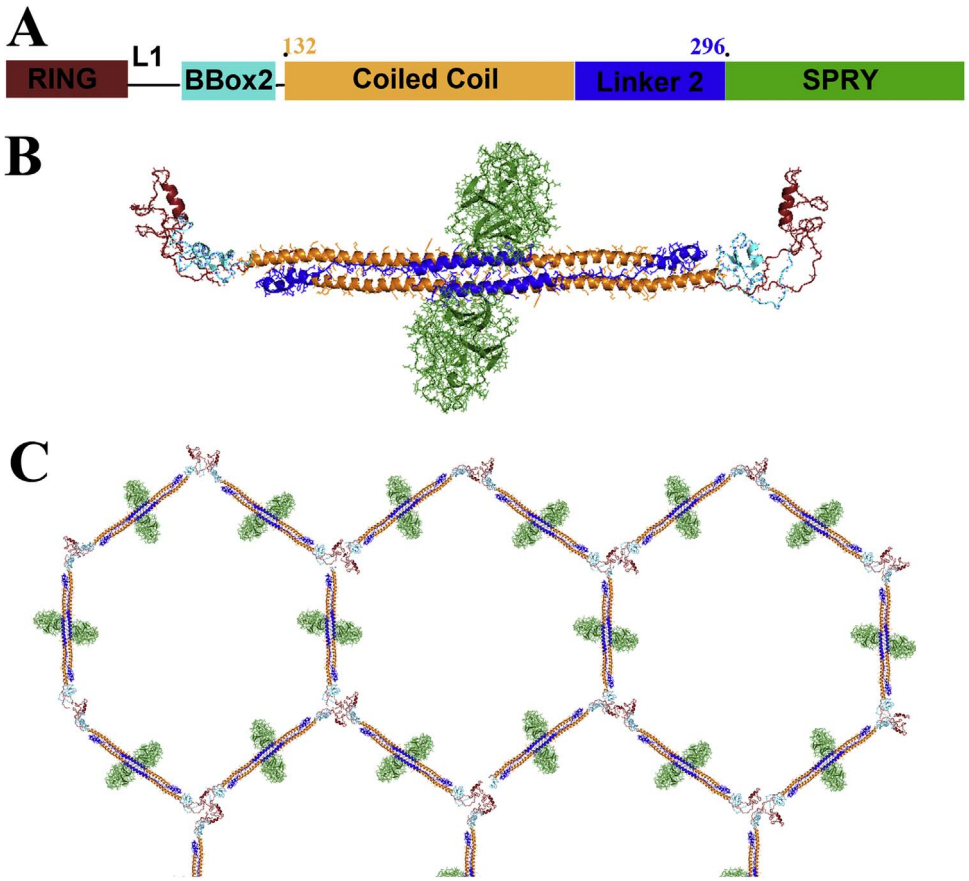

Fig. 1. Structural organization of TRIM5α assemblies: A. The domain structure of TRIM5α, with color coded domain structure used throughout the manuscript. B. Putative structure of TRIM5α dimer, assembled from a homology model of the CC-L2 dimer of rhTRIM5α and the structures of individual domains (Abe et al., 2007; Biris et al., 2012; Goldstone et al., 2014). C. Putative domain structures within hexagonal TRIM5α assembly (Ganser-Pornillos et al., 2011).

and BBox2 domains, exhibited the ability to disrupt CA tubes in vitro, suggesting that the minimal components of TRIM5α which induce the abortive disassembly of the viral core are located in the CC-L2-SPRY fragment of TRIM5α (Zhao et al., 2011). Although the mechanism underlying the disruption of assembled CA by the CC-L2-SPRY fragment was not determined, in the absence of enzymatic activity, one possibility is that dynamic changes in the conformation of these domains cooperatively induce CA disassembly. Consistent with this hypothesis, the recently published structure of the TRIM5α dimer failed to resolve a stretch of residues in the L2 region (Goldstone et al., 2014) which our studies have found to regulate the ability of rhTRIM5α to restrict HIV-1 infection (Sastri et al., 2014, 2010). To test the hypothesis that this region undergoes dynamic conformational changes, we performed single-molecule Förster Resonance Energy Transfer (smFRET) experiments to monitor conformational changes in the CC-L2 dimer. Because the resonance energy transfer between donor and acceptor fluorophores is governed by the interfluorophore distance, smFRET is a powerful method to precisely measure conformational changes which occur in a protein. We observe that the WT rhTRIM5α dimer exhibits substantial conformational variability, exchanging among at least three conformations. Moreover, mutants which exhibited altered restriction exhibited altered occupancy of these FRET states as well as altered ability to transition between the states. Collectively, these results reveal that the rhTRIM5α dimer undergoes dynamic conformational changes and suggest a model where transitions between individual conformations might account for the ability of TRIM5α to induce the disassembly of CA assemblies.

2. Materials and methods

2.1. Recombinant DNA

To generate 6xHis-tagged CCL2 peptides, the CCL2 fragments WT rhTRIM5α and its L2 mutants (residues 132–296 of the full length protein) were cloned into the pET-15b vector by using the NdeI and BamHI restriction sites. To introduce a C-terminal cysteine on the CCL2 peptides, primers were generated against the C-terminal end of the CCL2 gene fragment containing the codon for cysteine and mutagenesis was performed by PCR mutagenesis.

2.2. Protein expression and purification

Transformed BL21(DE3) cells were grown in 0.25 l of Luria broth containing 100 μg/ml carbenicillin (Invitrogen) until the optical density at 600 nm (OD_{600}) reached 0.6. The bacterial cultures were then induced to express WT or L2 mutant rhTRIM5α CCL2 peptides by adding 1 mM isopropyl β-D-1- thiogalactopyranoside (IPTG) (Invitrogen) and shaking the cultures for 4 h at 37 °C. To purify 6xHis-tagged CCL2 peptides, bacterial pellets were lysed in a solution containing 50 mM Na_2HPO_4, 500 mM NaCl, 10 mM imidazole, 1% Triton X-100, 0.5 mg/ml lysozyme (Sigma), 8 M Urea, and a protease inhibitor cocktail (PIC) (Roche), followed by sonication. The lysates were then centrifuged at 13,000 rpm at 4 °C for 30 min. The pellet was discarded, and the supernatant was incubated with Talon metal affinity resin (Clontech) at 4 °C for 1–2 h with gentle mixing to facilitate binding of the His-tagged proteins to the resin. The mixture was passed through a 2-ml Talon disposable gravity column (Clontech) twice. The flow-through was discarded, and the resin was washed with a buffer