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Binding of the rhesus TRIM5α PRYSPRY domain to capsid is necessary but not sufficient for HIV-1 restriction

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ABSTRACT

The PRYSPRY domain of TRIM5α provides specificity and the capsid recognition motif to retroviral restriction. Restriction of HIV-1 by rhesus TRIM5α (TRIM5α_{rh}) has been correlated to its ability to bind to the HIV-1 core, suggesting that capsid binding is required for restriction. This work explores whether the PRYSPRY domain of TRIM5α_{rh} exhibits an additional function besides binding to the HIV-1 core. Using our recently described structure of the PRYSPRY domain, we performed an exhaustive structure–function study of the surface and interior residues of the PRYSPRY domain. Testing retroviral restriction and capsid binding of an extensive collection of 60 TRIM5α_{rh} PRYSPRY variants revealed that binding is necessary but not sufficient for restriction. In support of this hypothesis, we showed that some human tripartite motif proteins bind the HIV-1 capsid but do not restrict HIV-1 infection, such as human TRIM6 and TRIM34. Overall this work suggested that the PRYSPRY domain serves an unknown function, distinct from the binding of TRIM5α_{rh} to the HIV-1 core, to block HIV-1 infection.

Introduction

Several newly discovered proteins that are endogenously expressed in primates show the ability to dominantly block retroviral infection and cross-species transmission by interfering with the early phase of viral replication (Best et al., 1996; Kirmaier et al., 2010; Sayah et al., 2004; Stremlau et al., 2004). Of particular interest are members of the tripartite motif (TRIM) family of proteins. The splicing variant a of TRIM5 from rhesus macaque (TRIM5α_{rh}) is a ~53 kDa cytosolic protein that potently restricts HIV-1 infection (Stremlau et al., 2004). Expression of TRIM5α_{rh} in mammalian cells blocks HIV-1 and other retroviruses soon after viral entry but prior to reverse transcription (Keckesova et al., 2004; Stremlau et al., 2004). The retroviral capsid protein is the viral determinant for susceptibility to restriction by TRIM5α (Owens et al., 2003). Studies on the fate of the HIV-1 capsid in the cytosol of infected cells have correlated restriction with a decreased amount of cytosolic particulate capsid (Diaz-Griffero et al., 2007a; Perron et al., 2007; Stremlau et al., 2006).

TRIM5α_{rh} is composed of four distinct domains: RING, B-box 2, coiled-coil and B30.2 (SPRY) (Reymond et al., 2001). The RING domain of TRIM5α_{rh} is an E3 ubiquitin ligase (Diaz-Griffero et al., 2006a; Kar et al., 2008; Kim et al., 2011; Langelier et al., 2008; Li et al., 2013; Lienlaf et al., 2011; Maegawa et al., 2010; Pertel et al., 2011; Yamauchi et al., 2008). The E3-ligase activity of TRIM5α is correlated to the ability of TRIM5α to block HIV-1 (Lienlaf et al., 2011). The B-box 2 domain of TRIM5α and other TRIM proteins, such as TRIM63, self-associates to forming dimeric complexes that are important for TRIM5α higher-order self-association and contribute to capsid binding avidity; these B-box 2 domain functions are essential for full and potent restriction of HIV-1 (Diaz-Griffero et al., 2007b, 2009; Ganser-Pornillos et al., 2011; Javanbakht et al., 2005; Mrosek et al., 2008; Perez-Caballero et al., 2005). The coiled-coil domain enables TRIM5α_{rh} dimerization (Kar et al., 2008; Langelier et al., 2008), which is critical for interaction of the B30.2 (SPRY) domain with the HIV-1 capsid (Sebastian and Luban, 2005; Stremlau et al., 2006).

The B30.2 (SPRY) domain provides the capsid recognition motif that dictates specificity to retroviral restriction (Nakayama et al., 2005; Sawyer et al., 2005; Song et al., 2005; Stremlau et al., 2005; Yap et al., 2005). Restriction of HIV-1 by TRIM5α_{rh} has been correlated to the ability of TRIM5α_{rh} to bind to the HIV-1 capsid, suggesting that capsid binding is required for restriction. An invariant patch on the human TRIM5α (TRIM5α_{hu}) protein has been described as being required for restriction of N-MLV but dispensable for capsid binding (Sebastian et al., 2009). By using a limited number of variants, these experiments showed that

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binding is necessary but not sufficient for restriction of N-MLV by TRIM5α_{hu} suggesting that the PRYSPRY domain has an additional function. To explore whether the PRYSPRY domain of TRIM5α_{rh} exhibits an additional function besides binding to HIV-1 capsid, we performed structure–function studies using our recently described structure of the PRYSPRY domain (Biris et al., 2012). Analysis of an extensive collection of PRYSPRY variants revealed two surface patches that are dispensable for binding but essential for retroviral restriction.

Results

Mutagenic analysis of the TRIM5α_{rh} PRYSPRY domain

Using the structure of the TRIM5α_{rh} PRYSPRY domain (Biris et al., 2012), we generated a collection of variants to test the hypothesis that the PRYSPRY domain exhibits an additional function besides binding to the HIV-1 capsid. As shown in Fig. 1, our mutagenesis studies focused on surface and internal residues of the TRIM5α_{rh} PRYSPRY domain (Table 1 and Fig. 1). This work explored most of the charge residues on the surface of the PRYSRY domain, and in cases where changes to alanine resulted in interesting phenotypes, we proceeded to change these amino acids to residues of opposite charge. For simplicity mutations PQIMY327AAAMA, NFNYC345AAAAA and PQIMY327AAAMA/NFNYC345AAAAA were called 327AAAMA, 345AAAAA and 327AAAMA/345AAAAA.

To evaluate expression, we stably transduced dog Cf2Th cells using retroviral vectors expressing HA-tagged mutant and wild type TRIM5α_{rh} proteins. Expression was evaluated by Western blotting using anti-HA antibodies (Figs. 2 and S1). As loading control, we blotted cellular extracts using anti-GAPDH antibodies. Most of the variants expressed equally well or better, when compared with to wild type TRIM5α_{rh} (Fig. 2 and Table 1). However, we also encountered a number of mutants, including D318E, Y364A, I391A, Y397A, S415A, F417A and R484A that were difficult to stably express in Cf2Th cells.

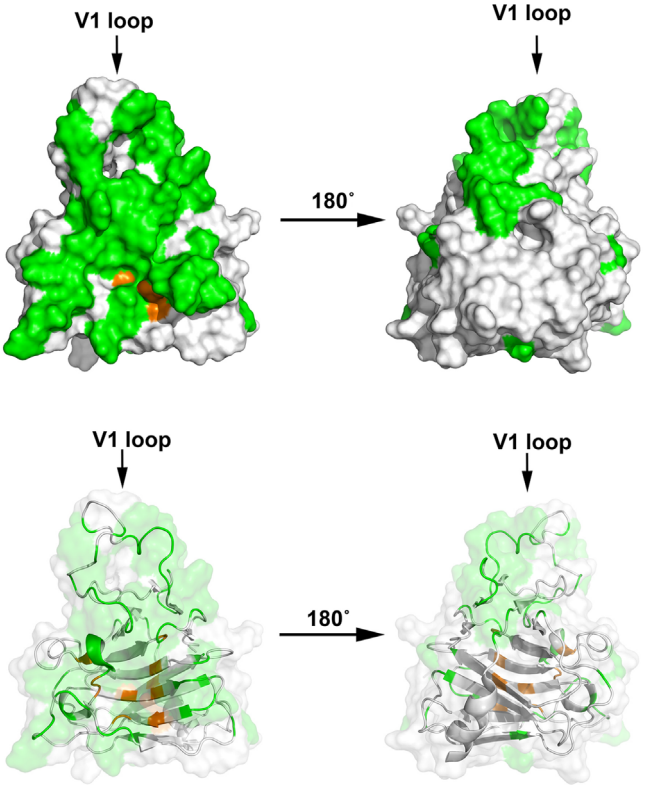

Fig. 1. Structure of the PRYSPRY of TRIM5α_{rh} showing the residues targeted in this study. The upper structures show the surface of the PRYSPRY domain of TRIM5α_{rh}. Surface and internal residues targeted for mutagenesis are shown in green and orange, respectively. The lower structures show a transparent surface revealing the polypeptide chain of the TRIM5α_{rh} PRYSPRY that similarly labels surface and internal residues targeted for mutagenesis in green and orange, respectively.

Ability of TRIM5α_{rh} PRYSPRY domain variants to restrict HIV-1

To test the ability of TRIM5α_{rh} PRYSPRY domain variants to block HIV-1 infection, we challenged Cf2Th cells stably expressing the different variants with increasing amounts of HIV-1 expressing GFP (HIV-1-GFP) as a reporter of infection. These experiments revealed that PRYSPRY domain mutants exhibit a variety of phenotypes ranging from full to the loss of restriction (Figs. 3 and S2). In Table 1, our PRYSPRY domain variants are ranked according to their ability to block HIV-1 infection.

Ability of TRIM5α_{rh} PRYSPRY domain variants to bind HIV-1 capsid

To test the ability of PRYSPRY domain variants to interact with the HIV-1 capsid, we tested the ability of the variants to bind in vitro assembled HIV-1 CA–NC complexes as previously described (Lienlaf et al., 2011). As in the restriction experiments, binding experiments revealed that the ability of PRYSPRY variants to bind HIV-1 capsid ranged from full binding to complete absence of binding (Figs. 4 and S3). In Table 2, PRYSPRY domain variants are ranked according to their ability to bind in vitro assembled HIV-1 CA–NC complexes. Of note, we found some variants, e.g. F417A and 327AAAMA/345AAAAA, that had completely lost the ability to bind in vitro assembled HIV-1 CA–NC complexes.

Binding of TRIM5α_{rh} to HIV-1 capsid is necessary but not sufficient for restriction

Previous experiments have demonstrated that binding of TRIM5α_{rh} to HIV-1 capsid is necessary for restriction (Diaz-Griffero et al., 2006b; Stremlau et al., 2006). For example, TRIM5α_{hu} does not bind the HIV-1 capsid, and is impaired in its ability to restrict HIV-1 (Li et al., 2006). In addition, mutations or deletions of TRIM5 or TRIMCyp that prevented binding to the HIV-1 capsid were unable to block HIV-1 infection, suggesting that binding is necessary for restriction (Diaz-Griffero et al., 2006b; Javanbakht et al., 2005; Perez-Caballero et al., 2005; Stremlau et al., 2006). To better understand the relationship between the ability of TRIM5α_{rh} to bind capsid and restrict HIV-1 infection, we plotted capsid binding as a function of the restriction abilities of different PRYSPRY variants. As shown in Fig. 5, the ability of TRIM5α_{rh} PRYSPRY variants to restrict HIV-1 requires binding; however, binding by itself is not sufficient for restriction since we found a collection of variants that showed wild type levels of binding to the HIV-1 capsid while losing their capacity to restrict HIV-1. These experiments suggested that the PRYSPRY domain is necessary for restriction but serves some function in addition to binding.

Careful analysis of variants that allow binding but lose restriction (binding $>$ 70% and restriction $<$ 40%) revealed two sets of residues that form surface patches important for restriction (Fig. 6): Surface patch 1(SP1) is composed of D318, K319 and R320 (Fig. 6A); Surface patch 2 (SP2) is composed of N326, P327, Q328, I329, M330 and Y331 (Fig. 6B). Even though SP1 and SP2 are located on the surface of the TRIM5α_{rh} PRYSPRY domain structure, we also found that changes in residues located inside the hydrophobic core, such as V405 and P483 depicted as the two black dots