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A human TRIM5α B30.2/SPRY domain mutant gains the ability to restrict and prematurely uncoat B-tropic murine leukemia virus

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ABSTRACT

Human TRIM5α restricts N-tropic murine leukemia virus (N-MLV) but not B-tropic MLV (B-MLV) infection. Here we study B30.2/SPRY domain mutants of human TRIM5α that acquire the ability to inhibit B-MLV infection prior to reverse transcription without losing the ability to restrict N-MLV infection. Remarkably, these mutants gain the ability to decrease the amount of particulate B-MLV capsids in the cytosol of infected cells. In addition, these mutants gain the ability to restrict SIV_{mac} and HIV-2 infection. B-MLV and SIV_{mac} infections were blocked by the mutant TRIM5α proteins prior to reverse transcription. Thus, the range of retroviruses restricted by human TRIM5α can be increased by changes in the B30.2/SPRY domain, which also result in the ability to cause premature uncoating of the restricted retroviral capsid.

Introduction

Following entry into cells, some retroviruses encounter dominant blocks to infection that act prior to reverse transcription (Cowan et al., 2002; Munk et al., 2002). For example, human immunodeficiency virus (HIV-1) is blocked in the cells of most Old World monkeys. Infection by the simian immunodeficiency virus of macaques (SIV_{mac}) encounters similar restrictions in New World monkeys (Hofmann et al., 1999). These restrictions are mediated by TRIM5α, a tripartite motif protein with a RING, B-box 2 and coiled-coil (CC) domain, as well as a carboxy-terminal B30.2/SPRY domain (Stremlau et al., 2004). The TRIM5α B30.2/SPRY domain is essential for retroviral restriction, and differences in the antiretroviral potency of TRIM5α from different species map to this domain (Ohkura et al., 2006; Perron et al., 2006; Sawyer et al., 2005; Song et al., 2005a; Stremlau et al., 2005; Yap et al., 2005).

Human TRIM5α ($TRIM5\alpha_{hu}$) potently restricts N-tropic murine leukemia virus (N-MLV) but not B-MLV infection (Hatziioannou et al., 2004; Keckesova et al., 2004; Perron et al., 2004). $TRIM5\alpha_{hu}$ partially inhibits equine infectious anemia virus (EIAV) and feline immunodeficiency virus (FIV) infections (Diaz-Griffero et al., 2007a,b). $TRIM5\alpha_{hu}$ does not efficiently restrict HIV-1 infection; however, a single amino acid change in its B30.2/SPRY domain ($TRIM5\alpha_{hu}$ R332P) converts $TRIM5\alpha_{hu}$ into a potent restrictor of HIV-1 and SIV_{mac} infections (Li et al., 2006; Stremlau et al., 2005; Yap et al., 2005). In contrast to the wild-type protein, $TRIM5\alpha_{hu}$ R332P efficiently binds HIV-1 capsid–nucleocapsid (CA–NC) complexes assembled *in vitro* (Li et al., 2006). Thus, variation in the surface of the B30.2/SPRY domain can modulate the specificity of retroviral restriction.

Changes in arginine 110 of the N-MLV capsid (CA) protein determine susceptibility to $TRIM5\alpha_{hu}$-mediated restriction. Changing this arginine residue to a glutamic acid residue, the corresponding amino acid from B-MLV, generates a virus that can partially overcome $TRIM5\alpha_{hu}$ restriction (Perron et al., 2004; Towers et al., 2000). Conversely, replacement of glutamic acid 110 of the B-MLV capsid with an arginine residue generates a virus that is susceptible to $TRIM5\alpha_{hu}$ restriction. Particulate N-MLV capsids in the cytoplasm of $TRIM5\alpha_{hu}$-expressing cells undergo a premature conversion to soluble capsid proteins (Perron et al., 2007). Decreases in the amounts of particulate cytosolic capsids have been observed for other retroviruses, including HIV-1, in cells expressing a restricting TRIM5α protein (Diaz-Griffero et al., 2007a,b; Stremlau et al., 2006). Thus, there is a good correlation between the restriction of a retrovirus and the premature uncoating of the viral capsid in the cytoplasm of the infected cell. However, other

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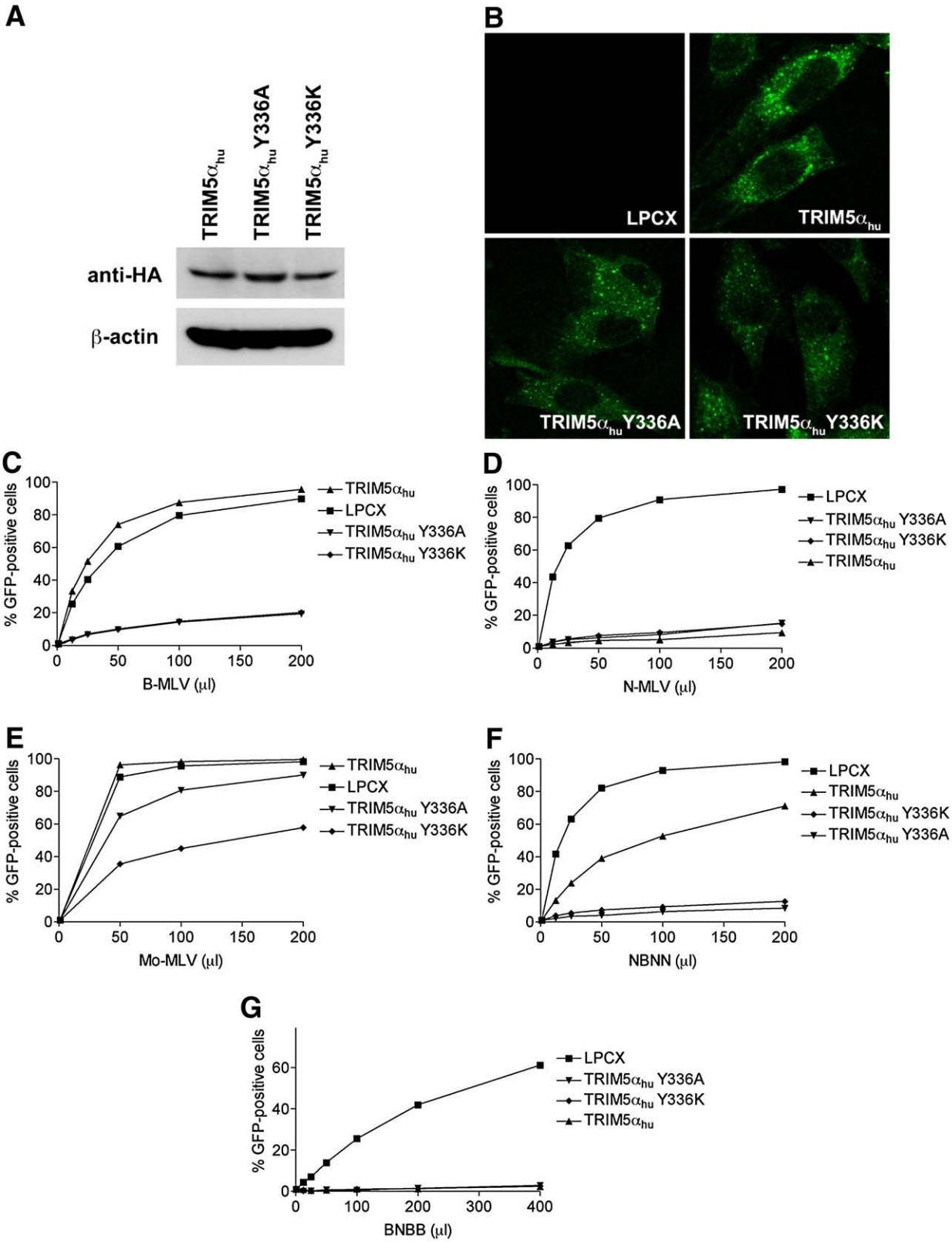

Fig. 1. Expression, localization and MLV-restricting ability of TRIM5α_{hu} Y336A and Y336K. (A) Wild-type, Y336A and Y336K TRIM5α_{hu} proteins were stably expressed in canine Cf2Th cells. Cell lysates were Western blotted and probed with antibodies directed against the HA epitope tag (top panel) or β-actin (bottom panel). (B) Cf2Th cells transduced with the empty LPCX vector or stably expressing the wild-type and mutant TRIM5α_{hu} proteins were fixed and stained using a fluorescein isothiocyanate-conjugated anti-HA antibody, as described in Materials and methods. Representative confocal microscope images are shown. (C–G) Cf2Th cells transduced with the empty LPCX vector or expressing the indicated wild-type or mutant TRIM5α_{hu} proteins were challenged with B-MLV-GFP (C), N-MLV-GFP (D), Mo-MLV-GFP (E), NBNN-GFP (F) and BNBB-GFP (G). GFP-positive cells were counted. Similar results were obtained in three independent experiments.