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Restriction of Retroviruses by a Cellular Factor TRIM5alpha

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Human immunodeficiency virus type 1 (HIV-1) is limited to humans and chimpanzees, whereas simian immunodeficiency viruses (SIV) naturally infect Old World monkeys. The replication of HIV-1 and SIV depends on host cell factors, some of which potentially govern the species tropism of the viruses. HIV-1 and SIV entry into target cells is dependent on host cell receptors, CD4 and chemokine receptors. These receptors bind the gp120 envelope glycoproteins of the virus, promoting virus attachment and fusion of the viral and cellular membranes. Following entry, retroviruses must execute a series of processes to establish a permanent infection of the host cell. These include the uncoating of the viral core, reverse transcription, nuclear entry of the viral DNA, and integration of the viral DNA into the host genome.

Retroviruses encounter dominant post-entry restrictions in the cells of particular species. HIV infection is blocked in the cells of Old World monkeys, whereas SIV infection is blocked in most New World monkey cells. These species-specific, post-entry restrictions share common features: 1) the block occurs prior to reverse transcription; 2) the viral determinant of the susceptibility to restrictioin is the capsid protein; and 3) the host cell restricting factor can be competed by virus-like particles containing proteolytically processed capsid proteins of the restricted viruses.

TRIM5α was identified in a random genetic screen as a cellular factor responsible for the early post-entry block to HIV-1 in Old World monkeys. TRIM5α is a member of the large family of tripartite motif proteins (TRIM), and contains RING, B-box, coiled-coil, and B30.2 domains. TRIM proteins often self-associate and form aggregates called nuclear or cytoplasmic bodies. Although TRIM proteins have been implicated in transcriptional regulation, cell division, antiviral activity, determination of cell polarity, and differentiation, the precise functions of most TRIM proteins remain to be determined.

Species-specific variation in TRIM5α was analyzed by amplifying, cloning, and sequencing primate *TRIM5* orthologs. Lineage-specific expansion and sequential duplication occurred in the TRIM5α B30.2 v1 region in Old World primates and in v3 region in New World monkeys. Substitution patterns indicative

of selection were observed bordering these variable elements. These results suggest that occasional, complex changes were incorporated into the TRIM5α B30.2 domain at discrete time points during the evolution of primates. Some of these time points correspond to periods during which primates were exposed to retroviral infections, based on the appearance of particular endogenous retroviruses in primate genomes.

To test the functional significance of the variation among the primate TRIM5α proteins, we established HeLa cells stably expressing different primate TRIM5α proteins by retroviral vector system and challenged the cells expressing different TRIM5α proteins with GFP-expressing recombinant viruses (HIV-1 or SIV) pseudotyped with VSV-G envelope. TRIM5α proteins from Old World monkey species restricted infection by HIV-1 whereas TRIM5α proteins from New World monkey species restricted infection by SIV. Thus, variation in TRIM5α proteins among primate species account for the observed patterns of post-entry restrictions in cells from these animals.

TRIM5α protein oligomerized into trimers, and the TRIM5α coiled-coil and B30.2 domains made important contributions to the formation of the trimers. Trimerization may allow TRIM5α to interact with the threefold pseudosymmetrical structure on retroviral capsid. To gain an understanding of how TRIM5α restricts HIV-1 infection, we investigated the ability of TRIM5α to interact in a specific manner with the HIV-1 capsid. We demonstrated that TRIM5α variants from Old World monkeys specifically associate with the HIV-1 capsid and that this interaction depends on the TRIM5α B30.2 domain. Human and New World monkey TRIM5α proteins associated much less efficiently with the HIV-1 capsid, accounting for the lack of restriction in the cells of these species. We established a novel “fate of capsid” assay to follow the fate of the retroviral capsid in the cytosol of newly infected cells and examined the effect of expression of a restricting TRIM5α protein on the particulate and soluble forms of the capsid. After infection, the expression of a restricting TRIM5α in the target cells correlated with a decrease in the amount of particulate capsid in the cytosol. Thus, TRIM5α restricts retroviral infection by specifically recognizing the capsid and promoting its rapid, premature disassembly.

Currently, we are exploring the cellular and molecular mechanism for the anti-HIV-1 activity of TRIM5α. The outcome of this study will lead to an improved animal model of HIV/AIDS pathogenesis and may reveal new antiviral targets.