

In defense of the cell: TRIM5 α interception of mammalian retroviruses

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In the kingdom of the cell, TRIM5 α is the arrow of Paris. The genomes of humans and mice attest to a parasitic onslaught of retroviral infections over the millennia (1, 2). In several reports in this issue of PNAS (3–5) and another in press (6), it has become apparent that an ancient and potent antiviral defense meets mammalian retroviruses that elude adaptive immune responses and transgress the cell membrane barrier. Seeking a strategic weakness in the virion coat, TRIM5 α targets invading retroviruses for destruction.

Whereas a recent breakthrough identified TRIM5 α as a HIV-1 restrictive factor expressed by rhesus monkey cells (7), these new findings reveal that human and nonhuman primate TRIM5 α can also neutralize other lentiviruses and murine leukemia virus (MLV), a distantly related gammaretrovirus (3–6). In addition, one group discovered that certain MLV isolates are also restricted by primate TRIM1 (3). In the space of a few months, a potentially vast intracellular antiviral network is rapidly coming to view.

Fv1: An Early Paradigm

Intracellular antiviral responses represent the last line of defense in preventing infection of a host organism by retroviruses. Evidence of a specific antiretroviral response was first provided by studies of MLV replication in mouse cells of different genetic backgrounds. Expression of distinct alleles of the mouse *Friend virus susceptibility factor-1* (*Fv1*) locus dictated susceptibility to infection by B- or N-tropic MLV (8–11). Prototypical alleles, such as *Fv1-B*, restrict infection by N-MLV at an early postentry step, whereas cells expressing *Fv1-N* restrict infection by B-MLV. *Fv1*-tropism is determined by a small number of residues in the MLV capsid protein, with a single change in residue 110 sufficient to confer sensitivity to *Fv1-B* or *Fv1-N* (12). *Fv1* is present in limiting amounts in mouse cells and can be overwhelmed by saturating amounts of challenge virus (13). Strikingly, *Fv1* is related to murine and human endogenous retroviruses and encodes a molecule similar to retroviral coat proteins (14).

Although *Fv1* is unique to mice, human cells also restrict infection by N-MLV. Restriction by human cells can be overcome by a high concentration of N-MLV,

suggesting the presence of a limiting dominant factor that has been referred to as Restriction factor 1 (Ref1) (15). Resembling *Fv1-B*-mediated restriction, Ref1 restriction of N-MLV can be circumvented by a change in amino acid 110 of capsid. Evolutionary pressure against N-MLV infection extends to cells from nonhuman primates, such as African green monkey (AGM). Provocatively, cells from different nonhuman primates also restrict infection by HIV-1 at an early, postentry stage, whereas simian immunodeficiency virus (SIV) isolated from cognate species is not blocked (16, 17).

Were independent restrictive mechanisms responsible for the N-MLV and HIV-1 infection blocks present in nonhuman primate cells? An accumulating case of circumstantial evidence hinted at a relationship between the two blocks. Similar to *Fv1*- and Ref1-mediated blocks, cells from New and Old World monkeys manifest a saturable block to HIV-1 infection, referred to by some groups as lentiviral susceptibility factor 1 (L_{v1}) (18, 19). Restriction of HIV-1 by L_{v1} in rhesus macaque cells was also found to map to HIV-1 capsid (19, 20). Introduction of the chimeric virus, whereas HIV-1 using an SIV capsid was immune to L_{v1}-mediated restriction. Although these similarities between N-MLV and HIV-1 restrictions were tantalizing, elegant experiments by Hatzioannou *et al.* (21) ultimately provided a more tangible link between restrictive mechanisms present in the primate cells of Old World monkeys. These researchers observed that preincubation of AGM cells with a saturating concentration of HIV-1 increased their sensitivity to infection by N-MLV. Reciprocally, preincubation of AGM cells with N-MLV permitted more efficient infection by HIV-1. In each circumstance, the initial virus dose appeared to act as a decoy to the restrictive factors present in the AGM cells. Could the same innate defense mechanism be responsible for intercepting these distinct mammalian retroviruses? The answer to this question seemed within grasp when the cytoplasmic body component TRIM5 α was identified as a dominant factor restricting HIV-1 infection of rhesus monkey cells (7).

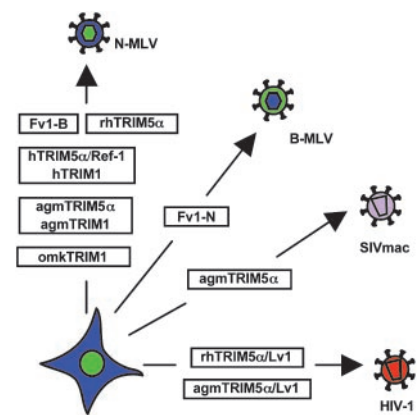


Fig. 1. Antiviral fusillade. Fv1, TRIM1, or TRIM5 α restriction of N-MLV (blue), B-MLV (green), SIV_{mac} (lavender), or HIV-1 (red). Boxes indicate species-specific restrictive measures targeting invading retroviral particles. h, Human; rh, rhesus macaque; agm, African green monkey; omk, owl monkey kidney.

TRIM5 α Neutralizes Mammalian Retroviruses

TRIM5 α has indeed been confirmed to be an essential component of the Ref1 restrictive activity observed in human cell lines (Fig. 1) (3–6). Expression of human TRIM5 α in otherwise permissive cells is sufficient to confer resistance to N-MLV but not B-MLV infection. Significantly, human cells transfected with small interfering RNA (siRNA) targeting endogenous TRIM5 α causes these cells to be nearly as sensitive to N-MLV infection as they are to B-MLV infection. Functional characterization of AGM and rhesus TRIM5 α variants recapitulates a broad restriction to N-MLV with no effect on B-MLV infection. Beyond N-MLV restriction, the different TRIM5 α variants recognize lentiviruses parasitic to other species. Although AGM TRIM5 α is ineffective against SIV isolated from AGM, its expression is sufficient to restrict HIV-1 or an SIV isolate propagated in macaques (4). Consistent with these data, TRIM5 α -specific siRNA treatment of AGM cells enhances their sensitivity to HIV-1 infection (5). Considered with results first observed using rhesus cells (7),

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it would appear that TRIM5 α from Old World primates accounts for Lv1. Finally, although human TRIM5 α is less effective at deterring infection by primate lentiviruses, human, rhesus, or AGM TRIM5 α can diminish infection by equine infectious anemia virus, a nonprimate lentivirus (4, 5).

Notably, the HIV-1 restrictive activity present in New World primates, such as owl monkeys, has yet to be identified. HIV-1 restriction in owl monkey kidney (OMK) cells can be pharmacologically manipulated through treatment with the immunosuppressant cyclosporine A (CsA), a competitive inhibitor of host cell cyclophilin A (CypA). CsA-treated OMK cells are 100-fold more permissive to HIV-1 infection than untreated cells are (22). Because HIV-1 capsid is known to interact with CypA (23), it has been postulated that this interaction in OMK cells may contribute to HIV-1 restriction. Understanding the relationship between CypA and HIV-1 restrictions in these cells will require a description of owl monkey TRIM5. In contrast, the HIV-1 restrictive properties of cells from Old World primates are significantly less affected by CsA treatment. Consistent with this observation, two reports (3, 6) indicate that HIV-1 capsid mutated to prevent CypA binding is still sensitive to AGM and rhesus macaque TRIM5 α .

Although a link between CypA and TRIM5 α remains to be uncovered, an exciting interaction between Fv1-N and TRIM5 α was detected by using human cell transfectants (5). Human TE671 cells restrict N-MLV infection by virtue of their endogenously expressed TRIM5 α (3, 5). These cells can be made resistant to B-MLV infection through the stable expression of Fv1-N, creating so-called TEN cells (18). Strikingly, when TEN cells are treated with siRNA to “knock down” endogenous TRIM5 α , Fv1-N restriction of B-MLV is lost (5). These data hint at a dependence of Fv1 antiviral function on a

TRIM-family protein from mouse. Given the common specificity of Fv1-B and human TRIM5 α for N-MLV capsid residue 110, such a relationship is not altogether surprising. Although there is no TRIM5 present in mice, there are a number of orthologs. Investigating potential interactions between murine TRIM-family proteins with N- and B-tropic MLV will likely provide insight toward the elusive mechanism of Fv1 restriction. Findings by Yap *et al.* (3) provide optimism in screening other TRIM family proteins for anti-retroviral properties. TRIM1 isolated from humans and nonhuman primates moderately restricts N-MLV infection. Sensitivity to TRIM1 restriction mapped to the same MLV capsid determinants that specify reactivity to TRIM5 α or Fv1 proteins. Defining shared TRIM1 and TRIM5 α regions required for antiviral function may help identify other restrictive factors from the TRIM family.

Future Studies

The expansion and diversification of TRIM family genes in metazoans suggests active and vital biological roles. Close to 40 genes are present in mouse and human. Even primate TRIM5 α isolates appear to be quite polymorphic. Although all primate TRIM5 α variants share a similar overall structure, significant differences were observed between intraspecies and interspecies isoforms. For example, AGM TRIM5 α contains a 20-aa insertion within a carboxyl-terminal domain when compared with the human isoform (3, 4). Notably, two AGM TRIM5 α isoforms even differ by 6 aa within this same region (3). It will be of interest to determine which changes within AGM TRIM5 α enhance its ability to recognize different retroviruses.

The preservation of TRIM5 α through the course of primate evolution has likely provided a significant barrier to interspecies retrovirus transmission. Uncovering a relationship between Fv1 and a mouse

TRIM5 α ortholog may provide an animal model in which the *in vivo* contribution of TRIM family genes to retroviral restriction can be directly examined. Similarly, siRNA knock-down of TRIM5 α in human or macaque primary CD4⁺ T cells could be used to measure the strength of restriction to N-MLV or HIV-1 infection, respectively, in these different species. Human polymorphisms in TRIM5 regulatory or coding sequences that correlate with differential susceptibility to HIV-1 infection or disease progression would also provide evidence of *in vivo* interactions of TRIM5 α with HIV-1.

A key unanswered question is the mechanism by which TRIM5 α neutralizes mammalian retroviruses. It has been hypothesized that TRIM5 α interdiction of retrovirions might disrupt an ordered uncoating process or sequester particles to a nonproductive infection pathway (7). Does TRIM5 α pierce the coat of the incoming retroviral? Early events in the retroviral lifecycle, particularly interactions with host proteins, have been notoriously difficult to monitor. Nonetheless, a novel assay to study the uncoating of avian retroviruses has recently been developed that, in principle, may be modified to study HIV-1 uncoating in the presence of TRIM5 α (24). The ability of the rhesus TRIM5 γ isoform to dominantly interfere with TRIM5 α antiviral function suggests that some domains may be modular (7). Thus, the delineation of TRIM5 α regions that specify virion recognition and those that promote antiviral function may allow for a more effective retargeting of human TRIM5 α . A superior understanding of human TRIM5 α function might eventually permit the therapeutic unshathing of this ancient defensive mechanism to help combat HIV-1 infection. If the rapid and clamorous characterization of the innate, antiviral factor APOBEC3G foreshadows the speed at which these questions will fall (25), this bold odyssey has already begun.

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