AIDS Reviews 2004;6:34-39

New Insights into the Role of Vif in HIV-1 Replication

Bärbel Schröfelbauer, Qin Yu and Nathaniel R. Landau
Infectious Disease Laboratory. The Salk Institute for Biological Studies, La Jolla, CA, USA

Abstract

HIV-1 and most of the other lentiviruses encode Vif (virion infectivity factor), an accessory protein that the virus requires to replicate in primary CD4+ T-cells and monocytes. The host cell factor with which Vif interacts was recently identified as APOBEC3G, a cytidine deaminase related to the RNA-editing enzymes. Identification of this key host protein has allowed for dramatic leaps in our understanding of how Vif functions. Vif prevents the encapsidation of APOBEC3G into HIV-1 virions during virus assembly. If not for Vif, the encapsidated APOBEC3G would damage the virus reverse transcripts, causing their degradation and closing the open reading frames of its genes. **(AIDS Rev 2004;6:34-9)**

Key words

Vif. HIV regulatory genes. HIV.

The Vif phenotype

Lentiviruses, except for equine infectious anemia virus, encode the accessory protein Vif (Virion infectivity factor). HIV-1 Vif is a 192 amino acid cytoplasmic protein that is expressed from a partially spliced mRNA late in the virus replication cycle[1,2]. It lacks obvious homology to known proteins. Vif was initially shown to be required for virus replication in primary cells[3,4], but subsequent surveys of transformed T-cell lines showed interesting differences in the ability of Δ*vif* HIV-1 to replicate[4-6]. Cells on which wild-type, but not Δ*vif* virus, replicated were classified as 'non-permissive'; those cells on which both viruses replicated were termed 'permissive'. Non-permissive cells include primary T-cells, macrophages and transformed T-cell lines such as PM1, Hut78 and CEM. Permissive cells include the T-cell lines SupT1, C8166 and CEMss and non-lymphoid cells such as HeLa.CD4. The phenotype is producer cell-dependent such that Δ*vif* virions produced from non-permissive cells are defective whether they are applied to permissive or non-permissive cells[4-6].

Δ*vif* virions produced in non-permissive cells are able to enter target cells, but abort replication prior to integration[7,8]. There is some question as to exactly where the block occurs, with reports of a block prior to or after reverse transcription[7-10]. A biochemical basis for the block to replication proved elusive. Analysis of the protein and RNA components of the defective virions on high resolution 2D protein gels revealed no difference between wild-type and Δ*vif* viruses produced in non-permissive cells using[10-13].

Cell-cell fusion experiments indicated that non-permissiveness was dominant over permissiveness[14,15]. That is, Δ*vif* virions produced from heterokaryons formed by the fusion of permissive and non-permissive cells were noninfectious. This finding suggested that non-permissive cells selectively express an antiviral factor that is neutralized by Vif.

Correspondence to:
Nathaniel Landau
Infectious Disease Laboratory
The Salk Institute for Biological Studies
La Jolla, CA 92037, USA
Phone: 858-453-4100, ext. 1334
E-mail: Landau@salk.edu

Discovery of the elusive host factor

The elusive cofactor CEM15 was identified by Sheehy, et al.[16] who used a subtractive cDNA cloning strategy to identify cDNAs that were specifically expressed in CEM but not CEMss, a closely matched pair with a clear difference in permissiveness. Expression of the gene by transfection of permissive cells rendered them non-permissive, proving the critical importance of the factor. In accord with the Vif phenotype, viral output from the cells was not affected.

CEM15 is identical to APOBEC3G (apoplioprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G), a member of the APOBEC family of cytidine deaminases that includes the cellular cytidine deaminases APOBEC1, APOBEC2, APOBEC3 and activation-induced deaminase (AID)[17,18]. In humans there are seven APOBEC3 genes (APOBEC3A-G) that lie in tandem on chromosome 22. Rodents have a single APOBEC3 gene[17]. No significant match has been found in *Saccharomyces cerevisiae, Drosophila melangogaster or Caenorhabditis elegans*[16]. APOBEC1, apoB editing catalytic subunit 1, the prototypical family member, catalyzes the deamination of C6666 to U of apoplioprotein B (apoB) mRNA, changing a glutamine codon (CAA) to a stop codon (UAA) to generate a truncated apoB48 protein[19-21]. The other family member that has been the object of recent attention is AID, a B-lymphoid protein that mediates the somatic hypermutation of immunoglobulin genes and stimulates immunoglobulin class switch recombination[17,22-26].

APOBEC3 proteins consist of an N-terminal catalytic domain fused to a pseudo-catalytic domain, joined by a linker to a similar repeat of both domains[17]. This structure suggests that it evolved from the duplication of a primordial cytidine deaminase. Each domain of the protein has a Cys-His Zn^{2+} coordination motif that forms the active site of the enzyme and is characteristic of cytidine deaminases. APOBEC genes are expressed with characteristic tissue specificity. APOBEC1 is primarily expressed in the small and large intestine, APOBEC2 in cardiac and skeletal muscle. AID is expressed in B-lymphocytes while APOBEC3G is expressed primarily in lymphoid and myeloid cell lineages[17].

Inactivation of HIV by APOBEC3G

In non-permissive cells, APOBEC3G is encapsidated into Δ*vif* HIV-1 virions in abundance[27-30]. The encapsidated enzyme does not interfere with virus assembly or release and does not block the ability of the virus to enter target cells. The vital clue as to the mechanism by which APOBEC3G interferes with virus replication was provided by an analysis of the reverse transcripts. Sequencing of the reverse transcripts of Δ*vif* HIV-1, but not wild-type virus, showed that they had numerous G→A mutations[27,31-34]. The G→A mutations were presumed to arise from cytosine deamination of the minus-strand of the reverse transcript. Because the minus-strand serves as the template for plus-strand synthesis, U nucleotides program the synthesis of a complementary A, resulting in a G→A mutation[27,31-34] (see model in Fig. 1). GG dinucleotides are attacked preferentially. Why are all of the mutations G→A and not C→T? There are two possible explanations. Either the minus-strand is specifically deaminated, or both strands are deaminated and then the plus-strand is repaired[27,31-34]. Cytosine deamination has two consequences for HIV-1 replication. First, it causes most of the reverse transcripts to be degraded prior to integration. This probably involves DNA repair enzymes that target uracil-containing DNA such as uracil DNA glycosylase (UDG) which removes uracil and AP endonuclease that cleaves at abasic sites. Second, it introduces numerous stop codons into the open reading frames.

Bioinformatic analysis of retroviral DNA sequences suggests that APOBEC3G has played a role in molding the HIV genome over evolution. Vif seems not to completely exclude APOBEC3G from virions, leading to continual low level of G→A mutation[27]. This was found to be the case for laboratory-generated wild-type virus produced in cells expressing transfected APOBEC3G. Several years earlier, interesting, but poorly understood observations were reported by Berhkout's group, who found that nucleotide content and codon usage in retroviral genomes is heavily skewed[35,36]. The genome of HIV-1 is A-rich, while other retroviruses, such as human T-cell leukemia virus type I (HTLV-I) and murine leukemia virus (MuLV) are C-rich. HIV-1 coding regions are 36% A and codon third base positions approach 60% A[35,36]. This 'A-pressure' could have been caused by reverse transcriptase errors or nucleotide pool imbalances, but pressure from APOBEC3G seems likely to have contributed.

Neutralization of APOBEC3G by Vif

APOBEC3G is encapsidated at high copy number in Δ*vif* virions, but was dramatically reduced in wild-type particles[27-30,37,38]. Functionally-inactive Vif mutants failed to block APOBEC3G encapsidation[28,37-39]. These findings provided a molecular explanation for the ability of Vif to neutralize the antiviral activity of APOBEC3G. Because the antiviral effect is mediated during reverse transcription by encapsidated APOBEC3G molecules, its exclusion from the virion by Vif prevents its antiviral activity (Fig. 1). This hypothesis was supported by co-immunoprecipitation experiments that showed that HIV-1 Vif and human APOBEC3G form a complex[27-30,37,40]. This finding does not prove a direct interaction of the two proteins. However, the species-specificity of the interaction (described below) is most easily explained by direct interaction of Vif with APOBEC3G.

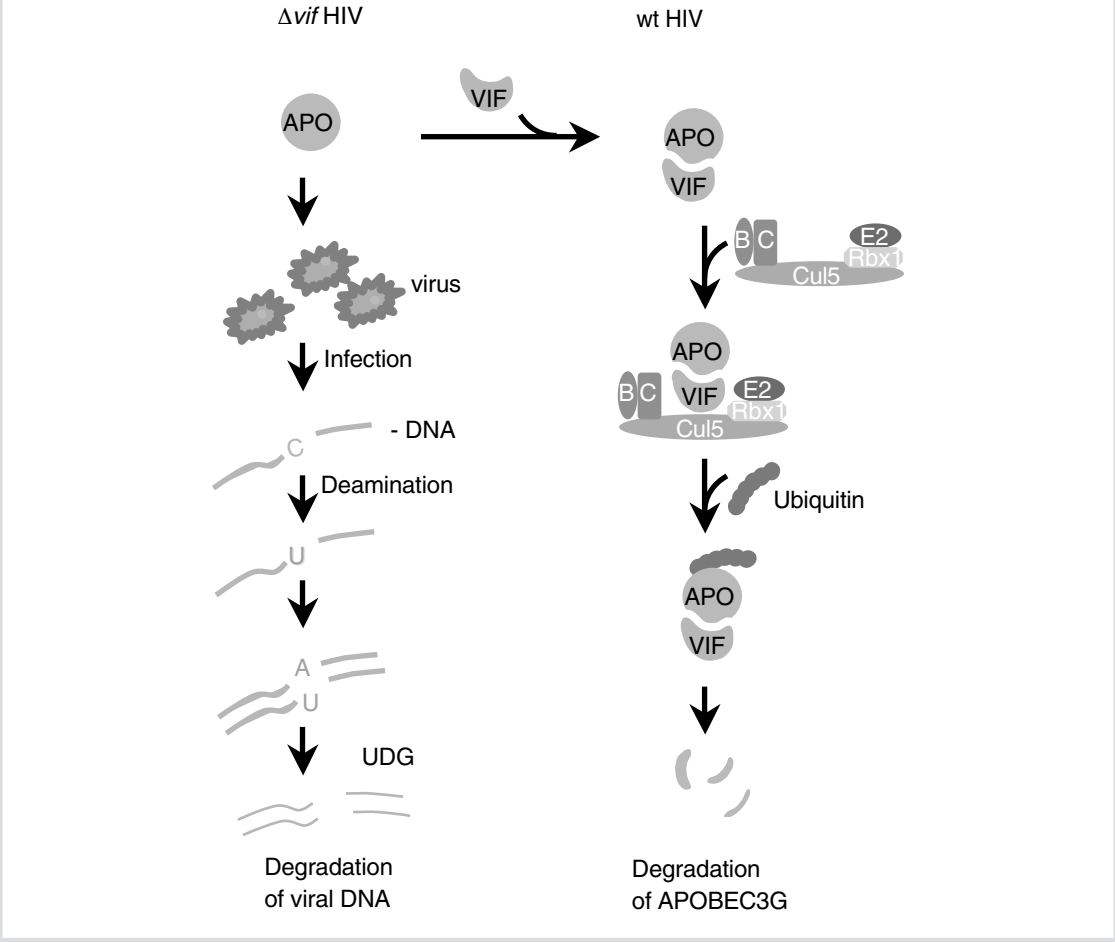

Figure 1. *Model for deamination of viral reverse transcripts in* Δvif *HIV-1 and Vif-induced degradation of APOBEC3G in wild-type virus. In* Δvif *virus, APOBEC3G is encapsidated during assembly. Upon a second round of infection, the encapsidated APOBEC3G deaminates cytosines in the minus-strand of the reverse transcript resulting in G→A mutations in the plus-strand. In wild-type virus, Vif binds to APOBEC3G and the complex associates with the SCF-like complex composed of elongin B/C, Rbx1 and Cul5. This results in ubiquitination of APOBEC3G which is then degraded by proteosomes.*

HIV-2 and SIV_{mac} Vif functionally substitute for HIV-1 Vif in terms of virus replication in human T-cells[41,42]. In contrast, SIV_{agm}, visna, feline and bovine immunodeficiency viruses were inactive in non-permissive human cells. In addition, SIV_{agm} Vif complemented Δ*vif* HIV-1 and SIV_{agm} produced in simian cells, while HIV-1 Vif was inactive. These findings are most easily explained by species-specific interaction of Vif and APOBEC3G.

AGM and rhesus macaque APOBEC3G differ from the human protein by about 30%, whereas APOBEC3G from chimpanzee is about 95% identical to human APOBEC3G[27]. All three-primate proteins were active against Δ*vif* HIV-1. Interestingly, AGM and rhesus APOBEC3G blocked the infectivity of wild-type HIV-1, indicating that HIV-1 Vif is unable to overcome the antiviral activity of these primate proteins. Similarly, mouse APOBEC3G blocked HIV replication and was resistant to Vif[27]. Conversely, SIV_{mac} Vif neutralized all of the APOBEC3Gs, while neither the human nor macaque protein was blocked by SIV_{agm} Vif. In summary, HIV-1 and SIV_{agm} neutralize APOBEC3G only from the species from which they are derived. In contrast, SIV_{mac} Vif neutralized all of the primate APOBEC3Gs[27]. SIV_{agm} Vif did not prevent human APOBEC3G encapsidation[27]. In addition HIV-1 but not SIV_{agm} Vif physically interacted with human APOBEC3G[43], suggesting that the interaction is critical for biological function (summarized in Fig. 2).

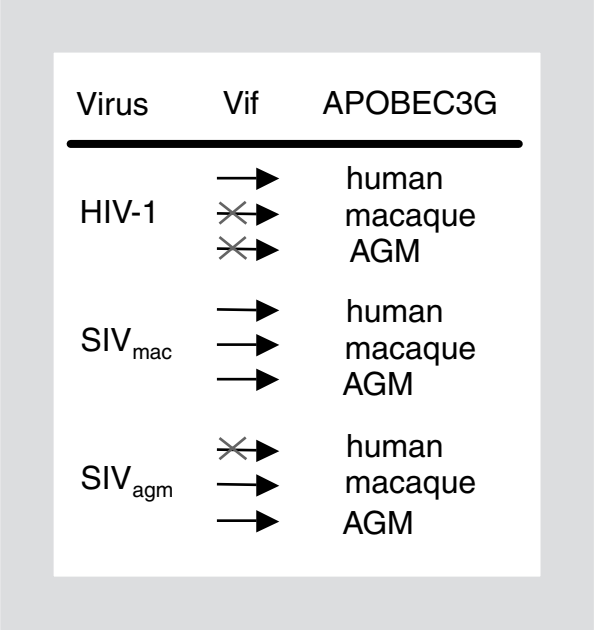

Figure 2. *The functional interaction of HIV-1, SIVmac and SIVagm with APOBEC3G. An arrow indicates that the viral Vif neutralizes the APOBEC3G.*

The species-specificity of the Vif:APOBEC3G interaction is caused by a single amino acid[44]. Exchange of Asp128 in human APOBEC3G with Lys128 of AGM APOBEC3G switched its interaction specificity for Vif. Conversely, the change of Lys128→Asp in AGM APOBEC3G resulted in a protein that was sensitive to HIV Vif and resistant to SIV_{agm} Vif. The switch in species-specificity was accompanied by a corresponding switch in the exclusion from virions and the physical interaction with Vif. These findings are most easily explained by a direct interaction of Vif with APOBEC3G.

The mechanism of Vif-induced APOBEC3G degradation

How does Vif exclude APOBEC3G from virions? Immunoblots on transfected cells showed that Vif caused a reduction in the steady-state level of APOBEC3G. APOBEC3G fragments were visible on the blots, suggesting that Vif may have induced the degradation of APOBEC3G[27-30]. Initial pulse-chase experiments by Mariani, et al.[27] failed to detect an effect of Vif on APOBEC3G half-life; however, subsequent findings from Marin, et al.[28] showed remarkably rapid degradation of APOBEC3G (2 min) that would have been missed in standard analysis. Other studies reported a half-life between 30 min and 4 h for APOBEC3G in the presence of Vif[29,30,37,38,40,45]. In addition, Stopak, et al.[29] found evidence for a second role for Vif in reducing the translation of APOBEC3G mRNA. Further evidence for a role of degradation was provided by the finding that proteasome inhibitors blocked the Vif-induced reduction of APOBEC3G levels and that Vif caused polyubiquitination of APOBEC3G[28-30,37,38,40,45].

The elegant findings of Yu, et al.[40] provided a molecular basis for Vif-induced APOBEC3G degradation. They noted that the highly conserved amino acid motif in Vif, SLQXLA, resembles a motif that targets proteins to an ubiquitin ligase. They further found that Vif coimmunoprecipitated with Cul5, elongins B and C and Rbx1, proteins that comprise an E3 ubiquitin ligase similar to the Skp1-cullin-F box (SCF) complex that targets proteins for ubiquitination and proteasomal degradation. Such complexes ubiquitinate a large number of cellular proteins, including those involved in cell-cycle regulation, signal transduction and transcription[46]. Vif can be considered an F-box-like protein that links the SCF complex to APOBEC3G (see model in Fig. 1).

Speculation and future perspectives

Although much new information has been unearthed regarding Vif function, important questions remain unanswered. How does APOBEC3G become encapsidated? Human APOBEC3G is encapsidated in HIV-1 and MuLV, which are quite divergent, and the mouse enzyme is also encapsidated in both viruses. This argues against a specific interaction with a viral protein, such as one of the Gag or Pol proteins, but does not rule it out (proteins such as RT have patches of conserved residues). In addition, an interaction with a specific viral protein seems unlikely since the virus could have easily escaped APOBEC3G by altering its protein-binding site. HIV-1 proteins are, for the most part, pretty malleable. The viral RNA would seem to be a likely means for APOBEC3G to gain access to the virus, and APOBEC3G has been shown to bind RNA (unpublished observation). While the RNA can change its sequence, it cannot fundamentally change its structure. APOBEC3G RNA binding would have to be specific to viral RNA; otherwise the enzyme would be titrated out on cytoplasmic cellular RNA. Specific binding could conceivably involve binding to secondary structures such as the RRE or TAR. Nevertheless, in preliminary experiments there did not seem to be an effect of mutations in the RNA packaging site on the amount of APOBEC3G encapsidated (unpublished observation).

The question of the resistance of simpler retroviruses to APOBEC3G deamination is unresolved. Mariani, et al.[27] and Harris, et al.[31] differed on the sensitivity of MuLV to APOBEC3G. Both groups studied model viruses *in vitro*. *In vivo*, it is clear that MuLV replicates in murine T-cells and that these express active APOBEC3G. The virus has no evidence of G→A hypermutation or suppression of APOBEC3G target sites and therefore must have a means of resisting the effects of the enzyme. MuLV encapsidated mouse APOBEC3G in co-transfection experiments, ruling out the possibility that the mouse virus fails to encapsidate the enzyme[27]. An attractive possibility is that MuLV uncoats differently than HIV-1, such that the encapsidated enzyme is released from the virion into the cytoplasm of the infected cell post-entry. Once released it would not be active against the virus, as demonstrated by the failure of APOBEC3G to act against incoming Δ*vif* virus.

Finally, what are the clinical implications of these studies? The Δ*32-ccr5* and *ccr5* promoter polymorphisms provide a clear precedent for the effect of host polymorphisms on disease pathogenesis. Polymorphisms in the APOBEC3G coding sequence could reduce the ability of Vif to bind APOBEC3G and allow it to escape inactivation. Polymorphisms in the promoter could alter expression. In tissue culture cells, sufficient expression of APOBEC3G overwhelmed Vif and blocked virus replication[27]. Donor variation could play a role in limiting virus replication *in vivo*.

Much remains to be learned about Vif and APOBEC3G. The experimental systems and reagents are in place, and further insight is surely around the corner.

Acknowledgments

Supported in part by NIH grants DA14494 and AI58864 and by the American Foundation for AIDS Research. NRL is an Elizabeth Glaser Scientist of the Elizabeth Glaser Pediatric AIDS Foundation.

References

1. Trono D. HIV accessory proteins: leading roles for the supporting cast. Cell 1995;82:189-92.
2. Emerman M, Malim M. HIV-1 regulatory/accessory genes: keys to unraveling viral and host cell biology. Science 1998;280:1880-4.
3. Sodroski J, Goh W, Rosen C, et al. Replicative and cytopathic potential of HTLV-III/LAV with sor gene deletions. Science 1986;231:1549-53.
4. Gabuzda D, Lawrence K, Langhoff E, et al. Role of vif in replication of HIV type 1 in CD4+ T lymphocytes. J Virol 1992,66:6489-95.
5. Strebel K, Daugherty D, Clouse K, Cohen D, Folks T, Martin M. The HIV 'A' (sor) gene product is essential for virus infectivity. Nature 1987;328:728-30.
6. Von Schwedler U, Song J, Aiken C, Trono D. Vif is crucial for HIV type 1 proviral DNA synthesis in infected cells. J Virol 1993;67: 4945-55.
7. Simon J, Malim M. The HIV type 1 Vif protein modulates the post-penetration stability of viral nucleoprotein complexes. J Virol 1996;70:5297-305.
8. Cohen E, Subbramanian R, Gottlinger H. Role of auxiliary proteins in retroviral morphogenesis. Curr Top Microbiol Immunol 1996;214: 219-35.
9. Goncalves J, Korin Y, Zack J, Gabuzda D. Role of Vif in HIV type 1 reverse transcription. J Virol 1996;70:8701-9.
10. Gaddis N, Sheehy A, Malim M. Comprehensive investigation of the molecular defect in vif-deficient HIV type 1 virions. J Virol 2003;77: 5810-20.
11. Fouchier R, Simon J, Jaffe A, Malim M. HIV type 1 Vif does not influence expression or virion incorporation of gag-, pol-, and env-encoded proteins. J Virol 1996;70:8263-9.
12. Hoglund S, Ohagen A, Lawrence K, Gabuzda D. Role of vif during packing of the core of HIV-1. Virology 1994;201:349-55.
13. Simm M, Shahabuddin M, Chao W, Allan J, Volsky D. Aberrant Gag protein composition of a HIV type 1 vif mutant produced in primary lymphocytes. J Virol 1995;69:4582-6.
14. Simon J, Gaddis N, Fouchier R, Malim M. Evidence for a newly discovered cellular anti-HIV-1 phenotype. Nat Med 1998;4: 1397-400.
15. Madani N, Kabat D. An endogenous inhibitor of HIV in human lymphocytes is overcome by the viral Vif protein. J Virol 1998;72: 10251-5.
16. Sheehy A, Gaddis N, Choi J, Malim M. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature 2002;418:646-50.
17. Jarmuz A, Chester A, Bayliss J, et al. An anthropoid-specific locus of orphan C to U RNA-editing enzymes on chromosome 22. Genomics 2002,79:285-96.
18. Harris R, Petersen-Mahrt S, Neuberger M. RNA editing enzyme APOBEC1 and some of its homologs can act as DNA mutators. Mol Cell 2002;10:1247-53.
19. Anant S, MacGinnitie A, Davidson N. Apobec-1, the catalytic subunit of the mammalian apolipoprotein B mRNA editing enzyme, is a novel RNA-binding protein. J Biol Chem 1995;270:14762-7.
20. Lau P, Villanueva H, Kobayashi K, Nakamuta M, Chang B, Chan L. A DnaJ protein, apobec-1-binding protein-2, modulates apolipoprotein B mRNA editing. J Biol Chem 2001;276:46445-52.
21. Yang Y, Sowden M, Smith H. Induction of cytidine to uridine editing on cytoplasmic apolipoprotein B mRNA by overexpressing APOBEC-1. J Biol Chem 2000;275:22663-9.
22. Muto T, Muramatsu M, Taniwaki M, Kinoshita K, Honjo T. Isolation, tissue distribution, and chromosomal localization of the human activation-induced cytidine deaminase (AID) gene. Genomics 2000;68:85-8.
23. Petersen-Mahrt S, Harris R, Neuberger M. AID mutates *E. coli* suggesting a DNA deamination mechanism for antibody diversification. Nature 2002;418:99-103.
24. Wedekind J, Dance G, Sowden M, Smith H. Messenger RNA editing in mammals: new members of the APOBEC family seeking roles in the family business. Trends Genet 2003;19:207-16.
25. Imai K, Slupphaug G, Lee W, et al. Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination. Nat Immunol 2003;4:1023-8.
26. Ta V, Nagaoka H, Catalán N, et al. AID mutant analyses indicate requirement for class-switch-specific cofactors. Nat Immunol 2003;4:843-8.
27. Mariani R, Chen D, Schrofelbauer B, et al. Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. Cell 2003;114:21-31.
28. Marin M, Rose K, Kozak SL, Kabat D. HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. Nat Med 2003;9:1398-403.
29. Stopak K, De Noronha C, Yonemoto W, Greene W. HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. Mol Cell 2003;12:591-601.

30. Sheehy A, Gaddis N, Malim M. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. Nat Med 2003;9:1404-7.
31. Harris R, Bishop K, Sheehy A, et al. DNA deamination mediates innate immunity to retroviral infection. Cell 2003;113:803-9.
32. Mangeat B, Turelli P, Caron G, Friedli M, Perrin L, Trono D. Broad antiretroviral defense by human APOBEC3G through lethal editing of nascent reverse transcripts. Nature 2003,424:99-103.
33. Lecossier D, Bouchonnet F, Clavel F, Hance A. Hypermutation of HIV-1 DNA in the absence of the Vif protein. Science 2003,300:1112.
34. Zhang H, Yang B, Pomerantz R, Zhang C, Arunachalam S, Gao L. The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. Nature 2003;424:94-8.
35. Berkhout B, Grigoriev A, Bakker M, Lukashov V. Codon and amino acid usage in retroviral genomes is consistent with virus-specific nucleotide pressure. AIDS Res Hum Retroviruses 2002;18:133-41.
36. Berkhout B, Van Hemert F. The unusual nucleotide content of the HIV RNA genome results in a biased amino acid composition of HIV proteins. Nucleic Acids Res 1994;22:1705-11.
37. Mehle A, Strack B, Ancuta P, Zhang C, McPike M, Gabuzda D. Vif overcomes the innate antiviral activity of APOBEC3G by promoting its degradation in the ubiquitin-proteasome pathway. J Biol Chem (in press).
38. Kao S, Khan M, Miyagi E, Plishka R, Buckler-White A, Strebel K. The HIV type 1 Vif protein reduces intracellular expression and inhibits packaging of APOBEC3G (CEM15), a cellular inhibitor of virus infectivity. J Virol 2003;77:11398-407.
39. Shindo K, Takaori-Kondo A, Kobayashi M, Abudu A, Fukunaga K, Uchiyama T. The enzymatic activity of CEM15/Apobec-3G is essential for the regulation of the infectivity of HIV-1 virion but not a sole determinant of its antiviral activity. J Biol Chem 2003;278:44412-6.
40. Yu X, Yu Y, Liu B, et al. Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. Science 2003;302:1056-60.
41. Simon J, Southerling T, Peterson J, Meyer B, Malim M. Complementation of Vif-defective HIV type 1 by primate, but not nonprimate, lentivirus vif genes. J Virol 1995;69:4166-72.
42. Reddy T, Kraus G, Yamada O, Looney D, Suhasini M, Wong-Staal F. Comparative analyses of HIV-1 and HIV-2 Vif mutants. J Virol 1995;69:3549-53.
43. Liu B, Yu X, Luo K, Yu Y. Influence of primate lentiviral Vif and proteasome inhibitors on HIV type 1 virion packaging of APOBEC3G. J Virol 2004;78:2072-81.
44. Schrofelbauer B, Chen D, Landau N. A single amino acid of APOBEC3G controls its species-specific interaction with Vif. Proc Natl Acad Sci USA (in press).
45. Conticello S, Harris R, Neuberger M. The Vif protein of HIV triggers degradation of the human antiretroviral DNA deaminase APOBEC3G. Curr Biol 2003;13:2009-13.
46. Kile B, Schulman B, Alexander W, Nicola N, Martin H, Hilton D. The SOCS box: a tale of destruction and degradation. Trends Biochem Sci 2002;27:235-41.