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Vif and Apobec3G in the innate immune response to HIV: a tale of two proteins

Melissa A Farrow &
Ann M Sheehy†

†Author for correspondence
College of the Holy Cross,
Department of Biology,
1 College Street,
Worcester, MA 01610,
USA
Tel.: +1 508 793 2255;
Fax: +1 508 793 2696;
asheehy@holycross.edu

It is now 26 years after the first published report on HIV, and the global epidemic continues unabated, with estimates of over 33 million people currently infected, worldwide. Development of targeted therapies aimed at perturbing the HIV life cycle can be achieved only with a detailed comprehension of the dynamics of virus–host interactions within the cell. One such critical virus–host interaction is the recently elucidated interplay between the viral Vif protein and the innate immune defense molecule Apobec3G. Apobec3G potently suppresses HIV replication, but Vif can alleviate this inhibition, rescuing viral infectivity. Early work describing the characterization of Vif and the cloning and identification of Apobec3G as an antiviral are discussed. Recent advances detailing the mechanisms of the Vif–Apobec3G regulatory circuit and our nascent understanding of Apobec3G endogenous function are also presented. Collectively, these studies have shed light on potential novel therapeutic strategies aimed at exploiting Apobec3G antiviral function to abrogate HIV replication.

Initial forays into the molecular details of HIV-1 revealed the presence of a short open reading frame, termed the *sor* gene, that was highly conserved among lentiviruses, thus implicating it as being potentially important in the HIV-1 viral life cycle. Further analysis of the *sor* gene product demonstrated that it was necessary for production of infectious virus from $CD4^+$ T-cell lines, which led to the protein being named viral infectivity factor (Vif) [1,2]. Vif-deficient (ΔVif) virions could be produced at normal levels from these cells, but they were unable to sustain a spreading infection.

Additional studies developed this finding and identified a subset of T-cell lines, termed nonpermissive cells, which were dependent upon Vif for production of infectious virus. Interestingly, Vif was dispensable for replication in other, permissive, cell lines [3,4]. This 'Vif phenotype' was determined to be producer-cell dependent as transcomplementation of Vif in the producer cell was sufficient to rescue viral infectivity, whereas expressing Vif in the target cell had no effect [1,4]. At the time, two potential explanations for this producer-cell phenotype were proposed. One line of thought suggested the presence of a Vif homologue in permissive cells while an alternative explanation proposed the presence of an inhibitory activity in nonpermissive cells that required Vif neutralization. An elegant series of heterokaryon experiments convincingly showed that nonpermissive cells expressed a dominant endogenous antiviral activity that was sufficient to inhibit HIV-1 ΔVif replication [5,6]. In a classic example of a viral counterattack to this innate antiviral activity, the HIV-1 Vif protein relieved this inhibition, allowing for propagation of a ΔVif virus. Thus, the basis of the Vif phenotype was explained, but the intriguing questions of who, what, when, where and how Vif was functioning awaited elucidation.

While the heterokaryon experiments provided a clear direction for further exploration of Vif function, the identity of the innate antiviral activity was elusive. Many groups utilized the permissive versus nonpermissive experimental system to gain a greater understanding of the expression, localization and post-translational modification of Vif, as well as expound upon the specific role of Vif in the viral life cycle. Ultimately, the innate antiviral activity present in nonpermissive cells was cloned by exploiting the dichotomy of two genetically related cell lines. The nonpermissive CEM T-cell line and its permissive clone, CEM-SS, were screened in a subtractive hybridization assay [7]. Potential cDNA candidates were first verified by differential expression screening and then validated by examining their antiviral activity in single-round infectivity experiments. This approach identified a clone, originally called *CEM15*, which met the criteria of differential expression and demonstrated potent anti-HIV activity. The differential expression of *CEM15* was expanded as it was found almost exclusively in nonpermissive T-cell lines and peripheral blood mononuclear cells, and absent from permissive cell lines. The antiviral

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activity of CEM15 was confirmed as stable expression of *CEM15* in retrovirally transduced CEM-SS cells was sufficient to convert CEM-SS from a permissive to a nonpermissive phenotype [7]. These experiments conclusively identified CEM15 as the innate viral restriction factor the field had been seeking. The cloning of *CEM15* not only answered the question of who, but also paved the way to answering the elusive questions of what, when, where and how Vif dictated the outcome of HIV replication.

Apobec3 family

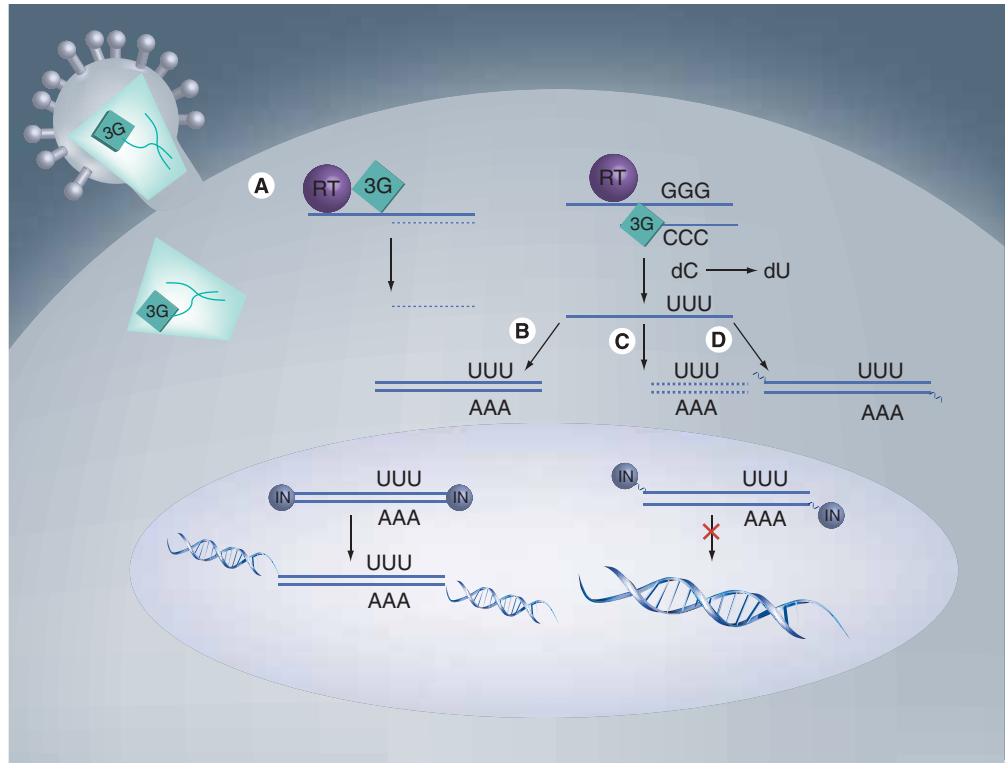
CEM15 was subsequently identified as *Apobec3G*, a member of the Apobec family of cytidine deaminases that catalyze C to U editing events, leading to G to A mutations, in RNA or ssDNA templates [8–13]. *Apobec3G* is one of seven Apobec3 family members found on chromosome 22 [14]. This family includes the founding member Apobec1, which deaminates *ApoB* mRNA in intestinal epithelial cells, as well as the lymphocyte-specific activation-induced deaminase, AID. AID functions in B cells where it plays a critical role in somatic hypermutation and class switch recombination by utilizing ssDNA as a substrate for deamination [8,15]. All members of the Apobec3 family contain at least one conserved cytidine deaminase active site (CDA), composed of the consensus sequence His-X-Glu-$X_{23–28}$-Cys-$X_{2–4}$-Cys [14,16]. Apobec3B, 3DE, 3F and 3G appear to have undergone further tandem duplication of the ancestral gene, giving rise to proteins with N- and C-terminal active sites bridged by a linker sequence [14]. As will be discussed later, these family members that contain multiple putative active sites function as antivirals against HIV-1, as well as other retroelements and viruses. There are several family members, including Apobec3A, 3C and 3H that contain a single CDA domain; while antiviral functions have been ascribed to both Apobec3A and 3C against adeno-associated virus and Simian immunodeficiency virus (SIV; SIVmac and SIVagm), respectively, in general, these family members primarily exhibit suppressive activity against endogenous retroelements, leading to their proposed anointing as 'guardians of the genome' [8,17,18]. Apobec3H, while a robust antiviral in primates, to date, does not have an identified antiviral function in human cells [19]. Finally, an important feature of this family of potent editors is their tightly controlled tissue-specific gene expression. Apobec3G is expressed in cells of the myeloid and lymphoid lineage, but details of its endogenous function remain undefined. This strict regulation is likely essential, preventing rampant, nonspecific editing events that may damage the genome [20].

Antiviral function of Apobec3G

Following the identification of Apobec3G, the Vif field was reinvigorated and a flurry of meticulously detailed publications examined the mechanism of Apobec3G antiviral function. Coupling previous observations that implicated defective postentry events, and the identification of Apobec3G as a DNA mutator, several groups quickly determined that Apobec3G mutated nascent viral cDNA transcripts during the reverse transcription process [9–12]. Upon entry of the viral core into the target cell, HIV undergoes a critical reverse transcription event to convert its ssRNA genome into dsDNA that can be integrated into the host cell genome. At several stages of reverse transcription, ssDNA intermediaries exist. These ssDNA molecules serve as substrates for deamination by Apobec3G. Certain areas of the genome, particularly the 3´ minus strand ssDNA intermediary, remain in a vulnerable single-stranded state for long periods and show a higher propensity for hypermutation [13,21,22]. By inducing hypermutation in the nascent viral cDNA transcripts, Apobec3G effectively renders the viral genome so unstable that reverse transcription is aborted or the sheer mutational load attenuates the resulting provirus. A third possible outcome is the recognition and degradation of uracil-containing DNA by virion incorporated host apurinic/apyrimidinic endonucleases [10–12,23]. Finally, it has recently been shown that Apobec3G activity results in aberrantly processed DNA that no longer serves as an efficient target for integration into the host genome [24,25]. Each of these possible events leads to a similar end point – abortive infection (Figure 1).

Comprehension of the mechanism of Apobec3G viral suppression was achieved at an astounding rate and our understanding of this innate line of defense against viruses has rapidly expanded. First, it was found that Apobec3G preferentially targets cytidine in the context of CC dinucleotides [12]. This intriguing observation, however, was inconsistent with the number and context of G to A mutations detected in clinical isolates, precipitating the identification of the highly homologous family member Apobec3F as an additional inhibitor of HIV [26–30]. Apobec3F is similar to Apobec3G in its Vif sensitivity and mediation of hypermutation of viral cDNA,

Figure 1. Apobec3G exerts pleiotropic effects during infection of the target cell.



Virion packaged Apobec3G can disrupt infection at several steps of reverse transcription and integration of the viral genome **(A)**. Apobec3G interferes with initiation and/or processivity of RT, leading to the synthesis of incomplete transcripts **(B)**. Apobec3G induces G→A hypermutation, leading to integration of a highly mutated genome **(C)**. Apobec3G deamination introduces uracil residues into nascent viral transcripts, leading to degradation by host enzymes **(D)**. Apobec3G interferes with integration of the viral genome by creating aberrantly processed ends that IN cannot interact with and/or directly inhibits IN function.
IN: Integrase; RT: Reverse transcriptase.

albeit at a lower level. The substrate preference of Apobec3F is for cytidine residues in a TC context, which explained the additional G to A mutations previously reported [26–28]. Together, Apobec3F and 3G lethally edit the HIV genome during reverse transcription. This identification of a second anti-HIV family member led to analysis of the other Apobec3 proteins as potential antivirals: Apobec3DE was identified as a Vif-sensitive inhibitor of HIV targeting CG for deamination while Apobec3B presented an interesting twist to the unfolding story [31]. Apobec3B behaves as an antiviral but, in contrast to the cytoplasmic Apobec3DE/F/G, it is localized to the nucleus, which may further explain its ability to resist Vif-mediated suppression [26,32]. The antiviral potency of Apobec3B appears tempered in comparison to that of Apobec3G, but its observable effectiveness against wild-type HIV makes it an incredibly attractive speculative target for therapeutic intervention.

The editing function of Apobec3G was the foundation of our understanding of the antiviral mechanism of the Apobec3 family and allowed for the delineation of additional antiviral family members. However, the story continued to evolve when it was reported that the editing and antiviral functions of Apobec3G are separable [33–35]. Using a panel of N- and C-terminal CDA point mutants, it was observed that the C-terminal CDA is necessary and sufficient for editing. Unexpectedly, further dissection indicated that Apobec3G mutants exhibiting compromised mutational capacity were not necessarily inactive in the exertion of an antiviral effect, indicating the presence of a second antiviral function. The mechanism for the editing-independent function of Apobec3G has not yet been precisely defined, but it appears that Apobec3G is capable of negatively impacting multiple stages of early viral replication, including the initiation and/or processivity of reverse transcription as well as

disrupting integration (Figure 1) [25,34–39]. Supporting the hypothesis of a multifunctional antiviral, several groups have reported that endogenous Apobec3G in resting target T cells can confer protection against incoming HIV infection in an editing-independent manner; negligible levels of hypermutated HIV were detected in these cells [40,41]. These findings suggest that Apobec3G is capable of disrupting the viral life cycle via an editing-independent mechanism. The relative contribution of either the editing-mediated or editing-independent mechanism to Apobec3G antiviral activity is currently unknown and likely to differ depending on the context of the infection [33,35,39,42,43]. It is now becoming clear that Apobec3G likely exerts pleiotropic effects on HIV.

Mechanism of Vif suppression of Apobec3G

While Apobec3G is able to potently inhibit HIV, the virus counters this innate cellular defense in a Vif-specific manner. A number of thorough studies implicating the proteasomal degradation pathway expedited our detailed understanding of the molecular mechanism of Apobec3G suppression [44–47]. Vif directs Apobec3G degradation by mediating its polyubiquitination. Vif serves as a scaffold, binding the substrate Apobec3G on the one hand and recruiting the Cul5–Elongin B–Elongin C–Rbx1 E3 ubiquitin ligase complex on the other, thereby mediating Apobec3G polyubiquitination and subsequent degradation [48]. Interestingly, the highly conserved SLQYLA motif in the C-terminus of Vif was identified as a novel SOCS box domain that binds Elongin C. Additionally, two conserved cysteines just outside the SOCS box domain were identified as interaction sites for Cul5. Together the SOCS box domain and conserved cysteines are sufficient to mediate interaction with the Cul5–Elongin B–Elongin C–Rbx1 E3 ubiquitin ligase complex [49–51]. With the active induction of Apobec3G degradation, Vif suppresses its antiviral function by simple elimination.

Our current understanding proposes that the primary function of Vif is virion exclusion of Apobec3G, and the degradation pathway is the most direct and clearly defined mechanism to achieve this strategy. However, it has been experimentally suggested and supported that Vif may also prevent virion incorporation of Apobec3G by alternative mechanisms, including subcellular sequestration. Residues within the Vif BC box are targets for phosphorylation, a modification occluding Elongin C interaction and disrupting the E3 ligase recruitment. However, phosphorylation of these residues does not completely ablate Vif function [51]. In addition, Vif can inhibit virion packaging of a degradation-resistant Apobec3G [52]. These data suggest that Vif could employ multiple means in Apobec3G suppression. Such alternatives may be important when Vif is located in a cellular microenvironment in which accessibility to cellular cofactors is limited or at a stoichiometry that does not favor induction of rapid Apobec3G turnover. By utilizing suppressive mechanisms that augment the degradation pathway, Vif could sequester or modify Apobec3G and prevent its packaging into the virus. While these mechanisms have not been precisely defined, it would not be surprising if Vif exploited multiple pathways to achieve its critical goal of virion exclusion of Apobec3G. Further clarification of the mechanism(s) of Vif-mediated virion exclusion of Apobec3G has distinct implications for therapeutic intervention.

HIV-1 virion packaging

The packaging of Apobec3G into the HIV-1 virion is essential to its antiviral activity, and Vif-mediated inhibition of this packaging is critical for the production of infectious virions. These observations raise the question of how Apobec3G successfully accesses the assembling virion. Many components, both cellular and viral, accumulate at the virus budding site during assembly. Among these required elements are the Gag polyprotein, which is the most abundant, and the HIV RNA genome. Both of these have been implicated in Apobec3G packaging. It has been convincingly established that Apobec3G packaging is dependent upon an interaction with the nucleocapsid domain of Gag and this interaction is facilitated by an RNA bridge [53–56]. However, the identity of that RNA bridge, whether it is cellular or viral, is formally still an open question. Recent work highlighting critical residues in an identified Apobec3G packaging domain may help to resolve the identity of the RNA bridge [57]. Understanding the precise steps involved in virion incorporation of Apobec3G could shed light on novel methods aimed at subverting Vif-mediated virion exclusion to enhance Apobec3G packaging into wild-type HIV, thus effectively terminating its replication.

The specific packaging of Apobec3G into the virion has led to intriguing speculation that the virus may have evolved a mechanism to package Apobec3G and utilize its mutator activity to

drive viral evolution. Prior to the discovery of Apobec3G, the genetic variability of the HIV genome observed in patient samples and tissue culture settings was attributed to the error-prone reverse transcriptase enzyme [58]. It was long believed that reverse transcriptase would randomly introduce errors into the genome and that some of those would be positively selected, explaining the inevitable appearance of immune escape epitopes and drug-resistance mutations [59]. However, closer examination of viral quasispecies suggests that in addition to the random mutations introduced by reverse transcriptase, there is a high frequency of specific mutations, particularly G to A hypermutations [59]. The elevated frequency of observed G to A mutations might be attributable to Apobec3G. A recent study has indicated that natural variation in patient *vif* alleles can result in partially active Vif proteins that incompletely neutralize Apobec3G or selectively suppress Apobec3F or 3G [60]. The authors propose that intermittent inactivation of Vif can contribute to Apobec3G-mediated viral sequence evolution. How the virus would usurp Apobec3G advantageously, but not allow for Apobec3G to exert antiviral inhibition, is unclear. Possible scenarios include modulation of the number and ratio of Apobec3G molecules packaged, or the introduction of subsequent compensatory mutations (within the viral genome) that convert an attenuated *vif* allele back to a functional one.

Apobec3G expression & subcellular localization

The proliferation of research aimed at understanding the antiviral function of Apobec3G has provided enormous insight into this innate immune defense molecule. However, identifying the endogenous function of Apobec3G has not been as tractable. This poses a major stumbling block for the development of antiviral therapies that would alter the Apobec3G landscape within the cell. A clearer understanding of the implications for the cell itself is essential before attempting the therapeutically relevant manipulation of Apobec3G. Such comprehension of the endogenous function of Apobec3G would undoubtedly assist in the development of therapies aimed at modulating its localization and antiviral function without compromising its cellular responsibilities. Ongoing studies aimed at elucidating the regulation and subcellular localization of Apobec3G continue to provide integral clues concerning its endogenous function.

A number of groups have focused on investigation of the regulation of Apobec3G expression, with both inducible (mitogenic stimulated and IFN-α responsive factors) and ubiquitous transcription factors, such as Sp1 and Sp3, being implicated in gene expression [61–65]. Further work in this area will be required to clearly define the specific pathways controlling Apobec3G expression in different cell types, but understanding the genetic regulation of *Apobec3G* expression may allow for controlled and directed upregulation of Apobec3G to overcome the Vif inhibition pathway. This strategy is supported by *in vivo* evidence highlighting the benefit of high levels of *Apobec3G*. Tantalizing clinical research has identified cohorts in which elevated expression of *Apobec3G* inversely correlates with HIV disease progression [66,67] or provides resistance to HIV infection in highly exposed seronegative individuals [68]. These preliminary reports suggest that increased levels of Apobec3G provide protection during exposure to HIV, as well as a prophylactic benefit if infection occurs.

In addition to the expression studies, the subcellular localization of Apobec3G is currently under intense investigation. A number of groups have shown that Apobec3G can be found associated with dormant mRNA in processing bodies or stress granules [69–71]. These ribonucleoprotein compartments are critical for the storage of mRNA, as well as regulation of mRNA metabolism and RNA-silencing pathways. The role Apobec3G plays in these RNA-regulating bodies is unclear, although it has recently been found that Apobec3G can alleviate miRNA inhibition of mRNA translation by redirecting miRNA targeted mRNA to polysomes rather than stress granules or processing bodies, suggesting a role in the regulation of translation pathways [72]. Additionally, Apobec3G has been detected in high molecular mass (HMM) ribonucleoprotein complexes. HMM-associated Apobec3G has been shown to protect against Alu retrotransposition events by recruiting Alu RNA into Staufen-containing granules, sequestering the RNA away from its replication machinery [73]. Clearly, Apobec3G resides in ribonucleoprotein complexes that are involved in RNA regulation on several levels.

The investigation of Apobec3G subcellular localization has also been approached from the perspective of antiviral activity. In resting $CD4^+$ T cells, fully active Apobec3G is partitioned into low molecular mass (LMM) ribonucleoprotein complexes where it seems to function as an antiviral against incoming HIV. In activated $CD4^+$

T cells Apobec3G resides in HMM complexes in a catalytically inactive state and is unable to exert a restrictive effect against incoming HIV. Treatment of these HMM complexes with RNase alleviates this block by releasing Apobec3G into a LMM complex, where it functions as an antiviral. This exciting discovery may explain why resting T cells, which predominantly contain LMM species, are resistant to HIV infection. Activation of the resting T cell by mitogenic stimulation also leads to recruitment of Apobec3G into the enzymatically inactive HMM complex and the cell acquires permissivity for HIV infection [40]. Interestingly, this partitioning phenomenon appears not to be restricted to T cells as monocytes and dendritic cells exhibit similar correlations between Apobec3G location and permissivity to HIV infection [74–76]. These observations in additional cell types that are vulnerable to HIV infection support the suggestion that manipulation of Apobec3G subcellular location may dramatically impact its antiviral capacity.

Intravirion RNase-mediated activation of Apobec3G also seems to be important. It appears that it is primarily newly synthesized Apobec3G that is actively packaged into the virion where it then associates with the viral RNA. However, upon this RNA association, catalytic activity of Apobec3G is blocked. The viral RNaseH activates Apobec3G by degrading the associated inhibitory RNA, liberating the fully catalytic Apobec3G [77]. It is suggested that its association with RNA mediates modulation of Apobec3G activity, both within the cell and the virion. A more detailed understanding of this Apobec3G–RNA interaction would provide additional information about Apobec3G function and regulation.

A more recent, somewhat unexpected finding regarding Apobec3G regulation, is the description of a novel inhibitory protein(s) in the cytoplasm of resting T cells that modulates Apobec3G enzymatic activity. In an attempt to correlate Apobec3G catalytic activity and antiviral function, the deamination capacity of endogenous Apobec3G in T cells was examined. Surprisingly, endogenous Apobec3G isolated from either primary T cells or T-cell lines was significantly less catalytically active than exogenously expressed Apobec3G in epithelial cell lines. Neither RNase, nor DNase treatment of endogenous Apobec3G was sufficient to alleviate the inhibition and induce enzymatic activity [41]. Insight into this novel, RNase-insensitive inhibitor could reveal additional pathways for catalytic activation of Apobec3G, which may further protect the cell from HIV invasion. Identifying this inhibitor would also provide critical information on the endogenous control of Apobec3G.

Vif–Apobec3G interaction

Apobec3G and Vif are involved in an intricate point–counterpoint battle that determines the outcome of HIV replication. Understanding how Vif and Apobec3G directly interact could allow for the development of inhibitors aimed at disrupting the interaction interface, rendering Apobec3G insensitive to Vif inhibition. Recent studies have delineated the binding sites in both Apobec3G and Vif that are critical for their interaction [57,78,79]. These studies show that the N terminus of both proteins is important for binding. Vif–Apobec3G interaction can be disrupted by small peptides or antibodies directed at the Vif interaction domain [78]. Also, residues in Apobec3G that mediate Vif-induced degradation have been identified [80]. The linker region between the N- and C-terminal CDA domains of Apobec3G, consisting of amino acids 105–245, is necessary and sufficient for Vif-mediated degradation. Interestingly, the authors identified Apobec3G fragments that can serve as dominant-negative inhibitors of Vif-induced degradation by disrupting Vif–Apobec3G binding (amino acids 1–156) or Vif-directed polyubiquitination of Apobec3G (amino acids 157–384). These identified amino acid residues can now serve as targets for the ablation of the Vif–Apobec3G interaction and Vif-directed degradation of Apobec3G.

Future perspective

Understanding the exact mechanistic details of the Vif–Apobec3G regulatory circuit will be essential for the design of targeted anti-HIV therapies. Since the identification of Apobec3G as a potent anti-HIV immune defense molecule, the field has moved forward at an expeditious rate, providing pivotal insight into the endogenous regulation of Apobec3G as well as its capacity as an innate viral restriction factor. However, additional work is needed to address key questions that will allow for the beneficial harnessing of Apobec3G antiviral capacity. The biggest challenge currently confronting the field is the comprehensive understanding of endogenous Apobec3G function. Continued efforts to more clearly define the role of Apobec3G in cellular RNA regulation will aid in the design of targeted therapeutic interventions of the Vif–Apobec3G interaction that avoid disruption of Apobec3G endogenous function(s). Additionally, the

intriguing observation of RNA-dependent partitioning of antiviral Apobec3G into LMM ribonucleoprotein complexes warrants further investigation. A more complete understanding of how LMM localized Apobec3G protects the cell from incoming HIV infection could reveal novel strategies that would utilize post-translational modulations to activate Apobec3G. Another key issue is defining the specific pathway(s) that regulate *Apobec3G* gene expression. Evidence supporting the strategy of elevating Apobec3G expression to overcome Vif inhibition is accumulating. If the precise transcription factor(s) controlling *Apobec3G* gene expression can be identified, perhaps they can be manipulated to effectively enhance Apobec3G expression in infected cells. Continued research into the structure of Apobec3G will reveal the precise Apobec3G residues that regulate sensitivity to Vif-mediated degradation. Identification of these residues may lead to the design of small molecule inhibitors that specifically target the Vif–Apobec3G interface and ablate interaction, emancipating Apobec3G from Vif. Finally, the editing-independent antiviral function of Apobec3G has tantalized the field for the past few years. Clearly defining this antiviral function will open new avenues for understanding and exploiting Apobec3G as an anti-HIV molecule. In the 5 years since the cloning of *Apobec3G*, enormous

Executive summary

Vif phenotype & the identification of Apobec3G

- Vif is necessary for the production of infectious HIV-1.
- Vif suppresses an innate antiviral factor in the producer cell.
- The innate antiviral factor was identified by a genetic screen that yielded the clone *CEM15*, subsequently identified as Apobec3G.
- Apobec3G is a member of a family of cytidine deaminases, of which the related family members Apobec3B, 3DE and 3F are also active against HIV-1.

Apobec3G antiviral functions

- Apobec3G hypermutates nascent viral cDNA transcripts by catalyzing a C to U deamination event, thereby inducing a G to A hypermutation in the viral genome.
- Hypermutation of the HIV genome disrupts the viral life cycle at the stages of reverse transcription and/or integration into the host genome.
- Integrated HIV genomes are attenuated by the sheer mutational load.
- Additional evidence suggests that Apobec3G also exerts an editing-independent antiviral function.
- The relative contribution and importance of the editing-mediated and -independent antiviral functions of Apobec3G remains an open question.

Apobec3G virion incorporation & mechanism of Vif suppression

- Apobec3G is incorporated into the HIV-1 virion via interaction with the viral protein nucleocapsid, which is mediated by an RNA bridge.
- Vif actively targets Apobec3G for proteasomal degradation by mediating polyubiquitination of Apobec3G.
- In addition to facilitating the degradation of Apobec3G, Vif excludes Apobec3G from the virion by alternative methods such as sequestration or modification.
- Further work is required to determine the precise mechanism(s) of Vif suppression of Apobec3G.

Apobec3G endogenous function

- Expression of Apobec3G can be modulated by inducible and ubiquitous transcription factors, but the specific pathways controlling *Apobec3G* expression await delineation.
- *In vivo* correlates suggest that elevation of *Apobec3G* expression will be an important therapeutic tool, further stressing the need to fully comprehend the transcriptional pathways that control endogenous expression of *Apobec3G*.
- Endogenous Apobec3G may be involved in mRNA metabolism and RNA silencing pathways.
- The subcellular localization of Apobec3G is critical to its antiviral capacity; fully active Apobec3G resides in low molecular mass ribonucleoprotein complexes.
- Continued investigation of the expression, localization and endogenous function of Apobec3G is necessary for the design of therapeutic intervention strategies targeting the Vif–Apobec3G circuit.

Vif–Apobec3G interaction

- The N-terminal regions of both Vif and Apobec3G are important for this protein–protein interaction.
- Amino acids 105–245 of Apobec3G are critical for Vif-mediated degradation.
- Additional studies are needed to further define the Vif–Apobec3G interface. Such information will facilitate the development of small molecule inhibitors aimed at ablating the Vif–Apobec3G interaction and perturbing Vif-directed degradation of Apobec3G.

progress has been made toward a thorough understanding of the Vif–Apobec3G regulatory circuit and how it influences HIV replication. The next 5–10 years should be equally as exciting, productive and insightful. Hopefully, we will refine and expand our current knowledge of Apobec3G expression, localization, regulation and function, yielding efficacious therapies designed to harness the power of an innate defense that can terminate HIV replication.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Affiliations

- *Melissa A Farrow*
College of the Holy Cross, Department of Biology, 1 College Street, Worcester, MA 01610, USA
Tel.: +1 508 793 3544;
Fax: +1 508 793 2696;
mfarrow@holycross.edu
- *Ann M Sheehy*
College of the Holy Cross, Department of Biology, 1 College Street, Worcester, MA 01610, USA
Tel.: +1 508 793 2255;
Fax: +1 508 793 2696;
asheehy@holycross.edu