Human Apolipoprotein B mRNA-editing Enzyme-catalytic Polypeptide-like 3G (APOBEC3G) Is Incorporated into HIV-1 Virions through Interactions with Viral and Nonviral RNAs*

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Apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G) is a host cytidine deaminase that is packaged into virions and confers resistance to retroviral infection. APOBEC3G deaminates deoxycytidines in minus strand DNA to deoxyuridines, resulting in G to A hypermutation and viral inactivation. Human immunodeficiency virus type 1 (HIV-1) virion infectivity factor counteracts the antiviral activity of APOBEC3G by inducing its proteosomal degradation and preventing virion incorporation. To elucidate the mechanism of viral suppression by APOBEC3G, we developed a sensitive cytidine deamination assay and analyzed APOBEC3G virion incorporation in a series of HIV-1 deletion mutants. Virus-like particles derived from constructs in which pol, env, and most of gag were deleted still contained high levels of cytidine deaminase activity; in addition, coimmunoprecipitation of APOBEC3G and HIV-1 Gag in the presence and absence of RNase A indicated that the two proteins do not interact directly but form an RNAse-sensitive complex. Viral particles lacking HIV-1 genomic RNA which were generated from the gag-pol expression constructs pC-Helper and pSyngp packaged APOBEC3G at 30–40% of the wild-type level, indicating that interactions with viral RNA are not necessary for incorporation. In addition, viral particles produced from a nucleocapsid zinc finger mutant contained ~1% of the viral genomic RNA but ~30% of the cytidine deaminase activity. The reduction in APOBEC3G incorporation was equivalent to the reduction in the total RNA present in the nucleocapsid mutant virions. These results indicate that interactions with viral proteins or viral genomic RNA are not essential for APOBEC3G incorporation and suggest that APOBEC3G interactions with viral and nonviral RNAs that are packaged into viral particles are sufficient for APOBEC3G virion incorporation.

Retroviral genomes, including that of HIV-1, undergo massive G to A substitutions in one or a few rounds of replication (1, 2). Because this high rate of G to A substitution (~2 mutations/100 nucleotides) is 1,000-fold greater than the rate of substitutions that arise during normal error-prone viral replication (~2 × 10⁻⁵ mutations/nucleotides/repllication), it is named hypermutation (1). Recent studies indicate that the HIV-1 Vif protein protects viral replication from a host restriction factor that induces hypermutation of the HIV-1 genome (3–6). Certain “restricted” cell lines and primary T cells suppress replication of HIV-1 genomes that do not express Vif (ΔVif virions), whereas other “permissive” cell lines allow replication of the ΔVif virions (7). Human apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G), first identified as CEM15 (8), is a dominant acting host factor that is responsible for the restricted cell phenotype (3–6, 9). APOBEC3G is a member of a family of cytidine deaminases that play a central role in generating somatic hypermutation and mRNA editing (10). Recent studies have shown that APOBEC3G expression in viral producer cells causes deamination of deoxycytidines in minus strand viral DNA to deoxyuridines, thereby resulting in G to A hypermutation (3–6, 9).

APOBEC3G must be packaged into virions to exhibit antiviral activity because its expression in target cells does not result in hypermutation or inhibition of viral replication. HIV-1 Vif overcomes the antiviral activity of APOBEC3G by inducing its rapid degradation through the proteosomal pathway, thereby preventing its incorporation into virion (11–15). It has also been reported that HIV-1 Vif reduces the efficiency of translation of APOBEC3G mRNA (16). We and others have shown recently that a single amino acid substitution in human APOBEC3G confers resistance to HIV-1 Vif (13, 17–19).

The mechanism by which APOBEC3G is incorporated into HIV-1 virions as well as other diverse retroviruses such as simian immunodeficiency virus, murine leukemia virus, and equine infectious anemia virus is unknown (4, 6, 20). The proteins and RNA components of these viruses share few common determinants that could be exploited by APOBEC3G to gain entry into viral particles. Furthermore, the high evolutionary potential of HIV-1 and other retroviruses should allow them to modify potential binding determinants quickly to prevent APOBEC3G incorporation. Instead, retroviruses have evolved the elaborate mechanism of encoding Vif to induce the proteosomal degradation of APOBEC3G. On the other hand, some protein determinants, such as the YXDD catalytic domain of reverse transcriptases or the zinc finger motifs of NC protein; elf3k, cytoplasmic translational initiation factor 3 subunit k; MA, matrix; NC, nucleocapsid; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; V; HIV-1 packaging signal; Vif, virion infectivity factor; VLP, virus-like particle; Gag, group antigen; HDV, HIV-1-derived vector.
proteins, are highly conserved in the orthoretroviruses and are essential for retroviral replication. Thus, these elements could potentially provide binding interactions that allow incorporation of APOBEC3G.

We have now developed a novel cytidine deaminase assay to determine APOBEC3G activity in virions. Deletion analysis of HIV-1 gag-pol expression constructs and their RNA content indicated that APOBEC3G incorporation into virions does not require a specific interaction with viral proteins or viral RNA. The results suggest that APOBEC3G is associated with viral and cellular RNAs that are specifically and non-specifically packaged into HIV-1 virions, respectively.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—pHDV-EGFP (kindly provided by D. Unutmaz, Vanderbilt University) (21), pNL4-3 (22), pdDNA-APO3G (11), a D128K mutant of APOBEC3G (13), pC-Help, and pMNC (kindly provided by D. Ott, AIDS Vaccine Program, SAIC-Frederick) (24), have been described previously. pGag expressed a full-length Gag protein that contained a FLAG tag at the C terminus. pGag, p43*, p41*, and pCA146* have been described previously (25, 26). pSYNGP, an HIV-1 cdon-optimized gag.pol expression construct, was obtained from Oxford Biomedica (UK) (27). The ΔMA fragment was generated by complete deletion of MA from pNL4-3 and insertion of the first 10 amino acids of Fyn at the N terminus of gag. pMA14CA129 was generated by an in-frame deletion in pC-Help that resulted in deletion from residue 15 of MA to residue 129 in CA and a Y130R substitution in CA. HIV-1 VLPs in PBS were treated with 15 units of RNase-free DNase I (Roche Applied Science) for 40 min in the presence of 2 mM MgCl2. After the addition of 500 μl of TBS (Tris-buffered saline; 50 mM Tris buffer, pH 7.6, 150 mM NaCl), the VLPs were centrifuged at 16,000 × g for 1 h at 4 °C, resuspended in 50 μl of RNase-free water and 3 μl of 20 mg/ml proteinase K (Invitrogen), and incubated at 55 °C for 30 min. Next, 200 μl of 5.8 mM guanidinium isothiocyanate and 10 μl of 20 mg/ml glycogen were added and incubated for 5 min at room temperature. RNA samples were precipitated with isopropl alcohol, centrifuged for 30 min at 4 °C, and RNA pellets were dissolved in 100 μl of water. Quantification of total RNA from VLPs was performed using the Ribogreen Quantitation Kit (Molecular Probes, Inc.). Quantification of HIV-1 RNA in VLPs was performed using reverse transcriptase-real time PCR as described previously (28). Briefly, cDNA synthesis was performed using random primers, and real time PCR was carried out in the presence of the U5-9 probe-primer set of HIV-1 (29).

**Immunofluorescence Staining of Transfected Cells**—293T cells were cultured on polyl-lysine-coated 12-mm-diameter coverslips (BD Biosciences) for 24 h prior to transfection. At 8 h post-transfection, cells were fixed with 2% paraformaldehyde and 2% sucrose in PBS and permeabilized with 1% Triton X-100 and 10% glycerol and stained with anti-c-Myc monoclonal antibody (9E10, Sigma) as primary antibody and Alexa Fluor 568-conjugated goat antibody to mouse IgG (L) (Molecular Probes) as secondary antibody. Cells were incubated sequentially with each antibody for 1 h and washed three times. All washes and antibody dilutions were done in PBS containing 1% bovine serum albumin (BSA). Coverslips were rinsed in deionized water and then mounted on glass slides by using FluoroGuard (Bio-Rad). Cells were visualized using a LSM510 Zeiss confocal microscope (Zeiss). The 材料内容包含有多种RNA的量以及其对活性的影响，具体的信息需要根据实验数据进行详细的分析。
Fig. 1. Scintillation proximity assay for APOBEC3G. A, schematic outline of the scintillation proximity assay for cytidine deamination. A scintillation proximity bead (gray sphere) that is coated with streptavidin (crescent), bound to a biotinylated oligonucleotide and hybridized to a template, is shown. Incubation of the template with HIV-1 VLPs containing APOBEC3G (APO) results in deamination of the deoxyuridines, converting them into deoxyuridines. The template-primer complex is incubated with Klenow fragment, resulting in [3H]dATP incorporation that is quantified. A parallel reaction is performed using Klenow fragment and [3H]dATP to normalize for the polymerization activity. B, cytidine deamination activity for the (CCCA)10 template from cells transfected with pcDNA-APO3G alone (APO only), pHDV-EGFP only (APO-HIV-1), and for various templates from cells transfected with both pcDNA-APO3G and pHDV-EGFP (APO+). C, correlation of APO activity with reaction time and enzyme concentration. The template-primer complex was incubated with VLPs derived from cells transfected with pHDV-EGFP with or without APOBEC3G using various amounts of p24 CA for 1, 2, or 6 h. Error bars represent the S.E. of two experiments.

RESULTS

To gain insights into the mechanism by which APOBEC3G is incorporated into retroviral particles, we developed a sensitive and quantitative cytidine deamination assay using scintillation proximity beads (Fig. 1A). Briefly, an oligonucleotide primer containing a 5′-biotinylated deoxynucleotide is hybridized to a template oligonucleotide containing deoxyuridines and deoxyadenines (e.g., (CCCA)10) and attached to scintillation proximity beads. The template-primer complex is incubated with HIV-1 VLPs containing APOBEC3G for 1–6 h at 37 °C. Next, the primer is extended using Klenow fragment in the presence of either a [3H]dATP, dGTP, and dTTP mixture or a [3H]dATP, dGTP, and dATP mixture. [3H]dATP is incorporated into the nascent DNA only upon conversion of deoxyuridines to deoxyuridines, providing a measure of the cytidine deamination activity. To control for efficiency of Klenow fragment-mediated DNA synthesis, incorporation of [3H]dATP is also determined. Incorporation of [3H]dATP or [3H]dATP in the extended primer brings the radiolabeled nucleotide in close proximity to the scintillation bead, resulting in a signal that can easily be quantified. An additional positive control reaction is performed in parallel using a (UUUA)10 template to determine the maximal [3H]dATP incorporation level, which was set to 100% and referred to as APO activity. The APO activity for experimental samples is expressed as a percentage of the [3H]dATP incorporation relative to the (UUUA)10 template.

To determine whether APOBEC3G and cytidine deamination activity is present in cell culture supernatants in the absence of HIV-1 proteins, we transfected 293T cells with pcDNA-APO3G plasmid and determined whether the culture supernatants contained cytidine deamination activity (Fig. 1B). A very low level of cytidine deamination activity (<2%) was detected, indicating that APOBEC3G release from 293T cells did not contribute significantly to the observed cytidine deamination activity. To determine which template sequences provided an optimal substrate for cytidine deamination, we compared the cytidine deamination activity after transfections with an HIV-1 gag-pol expression construct and an APOBEC3G expression plasmid on (CCA)13, (CCCA)10, and (CCCCA)8 templates (Fig. 1B). The results indicated that the (CCCA)10 template was the most efficient substrate for detection of cytidine deaminase activity; therefore, this template was used in all subsequent experiments.

Next, we determined how the quantity of VLPs and time of incubation of template with APOBEC3G correlated with the observed cytidine deamination activity (Fig. 1C). VLPs produced from cells transfected with HIV-1 gag-pol and APOBEC3G expression constructs were quantified by determining the amount of p24 CA levels by enzyme-linked immunosorbent assay; 0–45 ng of p24 CA associated with VLPs was incubated with the (CCCA)10 template-primer complex for 1, 2, and 6 h, and the extent of cytidine deamination was determined. The results indicated that the cytidine deamination activity increased as the amounts of CA associated with VLPs or time of incubation increased and leveled off at either high VLP input or longer incubation times (e.g., 12 ng of p24 CA for 6 h or 24 ng of p24 CA for 2 h). The data indicated that the cytidine deamination activity increased in a linear range of detection. Therefore, APOBEC3G activities were determined for ≤6 ng of p24 CA associated with VLPs for 2 h in subsequent experiments.

To determine whether APOBEC3G is packaged into HIV-1 VLPs specifically or nonspecifically, we compared the intracellular distribution and relative efficiencies of virion incorporation of APOBEC3G and cytoplasmic translational initiation factor 3 subunit k (eIF3k) (31). To compare the intracellular distributions of APOBEC3G and eIF3k, the plasmids that express each protein were transfected into 293T cells; the trans-
indicated that the APO activities in the transfected 293T cells activity (5 macrophages expressed lower levels of cytidine deamination cytidine deaminase activity. The results also suggested that Vif-permissive T cell line CEM-SS exhibited very low levels of virus-producing cells were harvested, and serial dilutions of the viral and cell lysates were analyzed by Western blotting using a monoclonal anti-c-Myc antibody. The samples contained 16, 8, 4, 2, and 1 μl of VLP lysate and 16, 8, and 4 μl of cell lysates, respectively.

To compare the level of virion incorporation of APOBEC3G with that of eIF3k, the plasmids expressing each protein were cotransfected along with an HIV-1 gag-pol expression construct. The amounts of both APOBEC3G and eIF3k were quantified in cell lysates as well as in VLPs produced from the transfected cells (Fig. 2B). The results indicated that APOBEC3G and eIF3k were expressed to equivalent levels in cell lysates; in contrast, there was an 8–16-fold enrichment of APOBEC3G in VLPs. This observation supported the hypothesis that an as yet unidentified mechanism results in the selective packaging of APOBEC3G in retroviral particles.

To elucidate whether transfection of 293T cells with APOBEC3G results in expression levels similar to the levels present in cells that are natural targets of HIV-1 infection, we compared the cytidine deamination activity in PBMCs, macrophages, an HIV-1 Vif-permissive T cell line (CEM-SS), and HIV-1 Vif-restricted T-cell lines CEMx174 and HUT78 (Fig. 3). The results indicated that transfections of 293T cells with 0.5 or 1.0 μg of APOBEC3G plasmid resulted in APO activities (40–50%) that were similar to those observed in PBMCs (20–30%) and HIV-1 Vif-restricted cell lines (25–40%). Previous studies with 0.5 or 1.0 μg of GFP expression plasmids have indicated that 50–80% of the 293T cells expressed the transfected marker genes (data not shown). As expected, the HIV-1 Vif-permissive T cell line CEM-SS exhibited very low levels of cytidine deaminase activity. The results also suggested that macrophages expressed lower levels of cytidine deaminase activity (5–10%) than PBMCs. Taken together, these results indicated that the APO activities in the transfected 293T cells were similar to those present in PBMCs and HIV-1 Vif-restricted T cell lines; thus, the VLP incorporation of APOBEC3G in transfected 293T cells was unlikely to be an artifact of APOBEC3G overexpression.

To determine whether interactions between HIV-1 Gag-Pol and APOBEC3G are necessary for incorporation of APOBEC3G into VLPs, we compared the cytidine deaminase activity present in VLPs produced from a series of HIV-1 gag-pol deletion constructs (Fig. 4A). We determined the APO activity in VLPs present in equivalent volumes of culture supernatants from transfected cells. The [3H]dATP incorporation was normalized to [3H]dTTP incorporation to minimize any differences resulting from variation in the efficiency of DNA synthesis. Culture supernatants from cells that were transfected with pcDNA-APO3G without any HIV-1 gag-pol expression constructs (APO only) exhibited very little cytidine deaminase activity (1,048 cpm of [3H]dATP incorporation/10,000 cpm of [3H]dTTP incorporation; average of two experiments and set to 1×). VLPs produced from wild-type HIV-1 (pNL43) generated 133-fold higher levels of [3H]dATP incorporation relative to the APO only control.

VLPs derived from various HIV-1 gag-pol deletion constructs exhibited cytidine deaminase activities and generated 19–92-fold higher levels of [3H]dATP incorporation relative to the APO only control. The results suggested that none of the Gag proteins (MA, N- and C-terminal portions of CA, p2, NC, p1, p6) or Pol proteins was essential for APOBEC3G incorporation into VLPs. The observed reductions in [3H]dATP incorporation for the HIV-1 gag-pol deletion constructs relative to wild-type were caused either by interactions between Gag proteins and APOBEC3G which facilitate APOBEC3G virion incorporation or previously observed decreases in VLP production (25).

To determine whether APOBEC3G and HIV-1 Gag proteins interacted directly, we cotransfected cells with pcDNA-APO3G and pGag, prepared extracts from the transfected cells, and performed coimmunoprecipitation analysis (Fig. 4B). Anti-c-Myc antibody was coupled to paramagnetic beads and used to immunoprecipitate the APOBEC3G proteins containing the c-Myc tag. The precipitated proteins were analyzed by Western blotting to determine the presence of APOBEC3G and HIV-1 Gag proteins using the anti-c-Myc and the anti-FLAG antibodies, respectively. To determine the potential role of RNA in interactions between APOBEC3G and HIV-1 Gag, we treated one aliquot of the cell lysates with RNase A to degrade the RNAs; conversely, we treated another aliquot of the cell lysates with an RNaseOut, an RNase inhibitor, to prevent degradation of RNA by RNases likely to be present in the cell lysate. The results indicated that APOBEC3G was immunoprecipitated from both aliquots of cell lysates, but the HIV-1 Gag was only coimmunoprecipitated from cell lysates that contained RNA. The results indicated that APOBEC3G was associated with RNA and that APOBEC3G and
HIV-1 Gag did not interact directly with each other; furthermore, APOBEC3G and HIV-1 Gag were in a complex with RNA that was sensitive to RNase A.

To determine whether interactions between HIV-1 genomic RNA and APOBEC3G play a role in the incorporation of APOBEC3G into VLPs, we determined the cytidine deaminase activity in VLPs produced from HIV-1 gag-pol expression constructs pSYNGP and pC-Help, which do not express any RNA containing the HIV-1 packaging signal (ψ/H9023) (Fig. 5A). Additionally, the HIV-1 RNA expressed from the codon-optimized pSYNGP lacks ψ/H9023 and has almost no homology to HIV-1 genomic RNA, further reducing the possibility that it will be packaged preferentially in VLPs. Different quantities of APOBEC3G expression plasmid were cotransfected along with the HIV-1 gag-pol expression constructs, and cytidine deamination activity was determined in equivalent amounts of VLP-associated material. The results indicated that when 0.5 µg of pcDNA-APO3G was transfected into cells to approximate the levels of cytidine deaminase activity present in PBMCs and Vif-restricted T cell lines (see Fig. 3), the VLP-associated cytidine deaminase activity in the absence of ψ/H9023-containing RNA was reduced to 30–40% of the VLP-associated activity in the presence of ψ/H9023-containing RNA. When 1.0 and 1.5 µg of pcDNA-APO3G were transfected and higher levels of APOBEC3G activity were present in the cells, the VLP-associated cytidine deaminase activity was increased further to 40–80%. This result indicated that the absence of HIV-1 ψ-contain-
into VLPs by up to 3-fold. We also determined the ability of an HIV-1 NC mutant (pCCHH), shown previously to be unable to package HIV-1 Ψ-containing RNA (28), to incorporate APOBEC3G into VLPs. The results indicated that the cytidine deaminase activity in VLPs derived from the HIV-1 NC CCHH mutant was similar to the VLPs derived from pSYNGP and pC-Help (reduced by up to 3-fold relative to the wild-type control). The reductions in VLP-associated cytidine deaminase activity in the absence of Ψ-containing RNA indicated that interactions between APOBEC3G and HIV-1 genomic RNA contributed to virion incorporation of APOBEC3G.

To verify that VLPs derived from the CCHH mutant of HIV-1 did not package HIV-1 genomic RNA efficiently, we isolated VLP-associated RNA and quantified the amounts of HIV-1 genomic RNA (Fig. 5B, black bars). The results, which were similar to those published previously (28), indicated that relative to the VLPs derived from the wild-type, the CCHH mutant VLPs contained less than 1% of HIV-1 genomic RNA. Even though the HIV-1 genomic RNA levels in VLPs were reduced 100-fold, APOBEC3G incorporation and VLP-associated cytidine deaminase activity were reduced only 3-fold. These results indicated that interactions between HIV-1 genomic RNA and APOBEC3G are not essential for virion incorporation of APOBEC3G.

Retroviral particles are known to package cellular RNAs in addition to viral RNAs nonspecifically (32). To determine the level of total RNA present in VLPs derived from the CCHH mutant, we determined the total RNA content of wild-type and CCHH mutant VLPs by using a Ribogreen quantitation assay (Fig. 5B, white bars). The results indicated that relative to the wild-type control, the amount of total RNA was reduced to 22% in VLPs derived from the CCHH mutant. Thus, the extent of APOBEC3G incorporation correlated with the amount of total RNA in VLPs, suggesting that interactions between APOBEC3G and cytoplasmic RNAs, including HIV-1 genomic RNA, play a role in packaging of APOBEC3G into HIV-1 VLPs.

**DISCUSSION**

The results of these studies indicate that interactions between APOBEC3G and viral proteins or viral RNA are not essential for virion incorporation of APOBEC3G and suggest that APOBEC3G interactions with viral as well as nonviral RNAs lead to its virion incorporation. A comparison of elF3k and APOBEC3G incorporation indicates that APOBEC3G is enriched in VLPs relative to elF3k. This observation is consistent with the existence of a specific mechanism for enrichment of APOBEC3G in VLPs but does not rule out the possibility that elF3k is specifically excluded from virions. Our results also indicate that APOBEC3G incorporation into VLPs was unlikely to be an artifact of overexpression of APOBEC3G by transient transfection because the cytidine deaminase activities in the lysates of cells transfected with 0.5 or 1.0 μg of pcDNA-APO3G were similar to those observed in PBMCs and the Vif-restricted cell lines CEMx174 and HUT78. It should be noted that transfections of cells with only 0.1 mg of pcDNA-APO3G, which approximated 50% of the APO activity in PBMCs (see Fig. 3), still resulted in a 16-fold enrichment of APOBEC3G into VLPs relative to elF3k, further supporting the view that APOBEC3G incorporation in these transfected cells was not the result of its overexpression.

The observations that APOBEC3G and HIV-1 Gag form a complex that is sensitive to RNase A indicate that APOBEC3G interacts with RNAs that also interact with HIV-1 Gag and support the view that APOBEC3G is incorporated into VLPs through interactions with packaged RNA. Our observation that VLPs lacking genomic RNA derived from pSYNGP, pC-Help, and the CCHH mutant of NC exhibited 3-fold lower cytidine deamination activity indicates that APOBEC3G interactions with genomic RNA play a role in APOBEC3G virion incorporation. The finding that VLPs derived from the CCHH mutant of HIV-1 gag-pol contained 100-fold less genomic RNA but only 3-fold less cytidine deamination activity indicates that interactions between HIV-1 RNA and APOBEC3G contribute to but are not essential for virion incorporation of APOBEC3G. Furthermore, the amount of total RNA in VLPs lacking genomic RNA (22%) correlated with the cytidine deaminase activity. These observations strongly imply that APOBEC3G is associated with viral and nonviral cytoplasmic RNAs and that specific and nonspecific packaging of these RNAs into VLPs, respectively, leads to virion incorporation of APOBEC3G.

The observations that APOBEC3G and HIV-1 Gag form a complex that is sensitive to RNase A indicate that APOBEC3G and HIV-1 Gag interact with the same RNAs and support the view that APOBEC3G is incorporated into VLPs through interactions with packaged RNA. Our data showed that APOBEC3G is incorporated into the VLPs derived from pSYNGP (27), which does not express HIV-1 envelope, Tat, Rev, Vif, Vpu, Vpr, or Nef, indicating that specific interactions with these viral proteins are not necessary for APOBEC3G virion incorporation. Analysis of the gag-pol deletion mutants also excludes specific interactions with most Gag-Pol proteins; however, the possibility that redundant binding motifs in different viral proteins are involved in APOBEC3G virion incorporation cannot be ruled out by this deletion analysis. In addition, a role for other components of the viral particles, such as the lipid envelope or incorporated host cell proteins, in virion incorporation of APOBEC3G cannot be excluded at this time. Furthermore, the deletion analysis also did not rule out possible interactions between APOBEC3G and 16 amino acids of CA. However, the observation that APOBEC3G is incorporated into several divergent retroviral particles that exhibit little homology in this portion of CA strongly implies that specific interactions with CA are not necessary for APOBEC3G virion incorporation (4, 6).

Currently, the physiological significance of APOBEC3G RNA interactions remains unclear. Although the substrate for APOBEC3G’s antiviral mechanism of action is single-stranded DNA, it was shown recently that APOBEC3G exhibits in vitro RNA binding activity (9) and perhaps utilizes certain nonviral RNAs as a substrate. The interaction of APOBEC3G with RNA may be a consequence of its two zinc finger motifs and general nucleic acid binding properties. Currently, the nature of the cytoplasmic RNAs that are associated with APOBEC3G also remains unclear.

Through the course of these studies, we have developed a sensitive and quantitative assay that detects cytidine deaminase activity in as little as 20 μl of culture supernatants containing VLPs. Unlike a cytidine deamination assay described previously, this assay does not require addition of purified uracil-DNA glycosylase or gel electrophoresis (33) and is more amenable to high throughput applications.

The conclusion that APOBEC3G interactions with viral and nonviral RNAs are responsible for its virion incorporation helps to explain why HIV-1 cannot simply mutate and modify structural determinants in viral genomic RNA or proteins to avoid APOBEC3G incorporation. HIV-1 and other lentiviruses have followed the evolutionary path of encoding the Vif proteins to stimulate proteosomal degradation of APOBEC3G. It will be of interest to determine whether retroviruses have also evolved other strategies to overcome APOBEC3G and the innate cellular defense mechanism of viral hypermutation.

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