Natural Polymorphisms and Oligomerization of Human APOBEC3H Contribute to Single-stranded DNA Scanning Ability

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**Background:** Human APOBEC3H is a mutation-inducing restriction factor for HIV-1 that exists as multiple haplotypes. APOBEC3H exists in humans as seven haplotypes (I–VII) with different cellular stabilities. Of the three stable APOBEC3H haplotypes (II, V, and VII), haplotypes II and V occur most frequently in the population. Despite APOBEC3H being a *bona fide* restriction factor, there has been no comparative biochemical characterization of APOBEC3H haplotypes. We characterized the ssDNA scanning mechanisms that haplotypes II and V use to search their ssDNA substrate for cytosine-containing deamination motifs. APOBEC3H haplotype II was able to processively deaminate multiple cytosines in a single enzyme-substrate encounter by using sliding, jumping, and intersegmental transfer movements. In contrast, APOBEC3H haplotype V exhibited diminished sliding and intersegmental transfer abilities but was able to jump along ssDNA. Due to an Asp or Glu at amino acid 178 differentiating these APOBEC3H haplotypes, the data indicated that this amino acid on helix 6 contributes to processivity. The diminished processivity of APOBEC3H haplotype V did not result in a reduced efficiency to restrict HIV-1 replication in single-cycle infectivity assays, suggesting a redundancy in the contributions of jumping and intersegmental transfer to mutagenic efficiency. Optimal processivity on ssDNA also required dimerization of APOBEC3H through the β2 strands. The findings support a model in which jumping can compensate for deficiencies in intersegmental transfer and suggest that APOBEC3H haplotypes II and V induce HIV-1 mutagenesis efficiently but by different mechanisms.

**Results:** Two APOBEC3H haplotypes were biochemically characterized.

**Conclusion:** An amino acid polymorphism between APOBEC3H haplotypes II and V alters their processivity, but not mutagenic ability. Dimerization is required for efficient processivity.

**Significance:** APOBEC3H has redundant processive mechanisms enabling haplotypes to efficiently restrict HIV-1 but by distinct mechanisms.

The human APOBEC3 (A3) family contains seven deoxycytidine deaminases that act as cellular restriction factors for a number of retrotransposons, retroviruses, and DNA viruses (1, 2). Four of the human A3 enzymes, A3D, A3F, A3G, and A3H, are able to restrict the replication of HIV-1 (referred to as HIV) by becoming encapsidated into budding virions and deaminating cytosines to uracils on the nascent (−)-DNA synthesized by HIV reverse transcriptase (3, 4). The uracil lesions result in transition mutations upon using the (−)-DNA as a template to synthesize the (+)-DNA (5–7). This results in decreases in HIV infectivity that are dependent on the number of mutations and the effect of the mutations on the viral proteins. Each deaminase has a preferred sequence context for deamination, but the minimal dinucleotides for recognition are 5′CC for A3G and 5′TC for A3D, A3F, and A3H (8–13).

The number of mutations that an A3 can introduce into the HIV proviral genome is mediated by the level of encapsidation into the viral capsid and the biochemical properties of the A3 (2, 14). The primary factor mediating A3 restriction ability is the HIV Vif (viral infectivity factor) protein (15). Vif acts as the substrate receptor of a CRL5 (Cullin RING ligase-5) E3 ligase complex to induce polyubiquitination and degradation of A3s relevant to HIV restriction (16–22). This greatly decreases the encapsidation levels of A3s into HIV virions but does not completely block A3 virion encapsidation because G to A mutations in a sequence context indicating the action of A3 enzymes are present in HIV genomes recovered from infected individuals (23). In the absence of Vif and despite equal encapsidation of some A3s into virions, there are differences in their efficiency of HIV restriction (e.g. A3G and A3F) (24, 25). These data demonstrate that there are biochemical differences in the enzymes that influence their activities as restriction factors (25). We have determined that the mechanisms that A3G and A3F use to scan the ssDNA substrate in search of the deamination motifs affect the number of induced mutations (25, 26). Synthesis of the proviral dsDNA is a dynamic process concurrently involv-

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2 The abbreviations used are: A3, APOBEC3; A2, APOBEC2; Z-domain, zinc-coordinating domain; nt, nucleotide(s).
Facilitated diffusion movements enable enzyme processivity, which is essential for the search process to locate the deamination motifs (2). A3 enzymes use sliding, jumping, and intersegmental transfer movements on ssDNA. The data demonstrate that A3H is a processive enzyme. A3H dimerization uses sliding, jumping, and intersegmental transfer to locate deamination motifs on ssDNA. The Glu-178 polymorphism of A3H haptotype V causes diminished sliding and intersegmental transfer but does not affect jumping. This difference in processivity did not affect HIV restriction ability. Further, we identified that the dimerization interface in A3H is mediated by the pairing of β2 strands from two monomers, and tetramerization can occur through the pairing of loop 7 from two dimers, as predicted could occur from a crystal structure of APOBEC2 (A2) with a 40-amino acid N-terminal deletion (45). The β2-β2 strand dimerization of A3H is required for efficient ssDNA scanning. The results demonstrate that A3H dimerization and Asp-178 is required for sliding and intersegmental transfer movements on ssDNA. The data combine to support a model in which A3H haptotypes II and V both induce HIV mutagenesis efficiently but by different mechanisms and suggest that jumping can compensate for deficiencies in intersegmental transfer.

**Experimental Procedures**

Cloning and Site-directed Mutagenesis—An A3H haptotype I clone (NCBI accession number BC069023) was obtained from Open Biosystems, and site-directed mutagenesis was used make A3H haptotype II (G105R/K121D/E178D) and haptotype V (G105R/K121D). A GST-A3H construct was subcloned from a pAcG2T vector (BD Biosciences) into a pFAST-bac1 vector (Life Technologies, Inc.) using EcoRI and NotI restriction sites. Mutants of A3H haptotype II (R44A/Y46A, Y111A/Y112A, D178K, and D178A) were made using the A3H haptotype II construct for site-directed mutagenesis (QuikChange site-directed mutagenesis protocol, Stratagene). The cDNA for A3H haptotypes II and V was also cloned into pcDNA3.1 with an HA

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The known function of the polymorphism of Asp/Glu at position 178 (41).

Despite the several unique aspects of A3H in comparison with the other A3 enzymes, there has been no in depth biochemical characterization of the most frequently occurring stable A3H haplotypes (II and V). This type of characterization is necessary to fully understand the determinants required for HIV restriction because A3H has several distinctive characteristics in the A3 family. A3s can be divided into two families based on the number of zinc-coordinating domains (Z-domains) (42). A3B, A3D, A3F, and A3G are deaminases with two Z-domains, whereas A3A, A3C, and A3H are deaminases with a single Z-domain. Notably, A3H is the only single Z-domain A3 that can restrict the replication of HIV (3). In addition, A3s have been classified into three categories based on the phylogenetic analysis of their Z-domains (Z1, Z2, and Z3), and A3H is the only Z3-type domain A3 enzyme (42). Part of the reason why A3H is able to restrict HIV is that it is the only single Z-domain A3 that oligomerizes in solution and on cellular RNA, which is a prerequisite for virion encapsidation (43, 44). However, there are no oligomerization models for single Z-domain A3 enzymes that have been verified as contributing to cellular function.

We undertook a biochemical characterization of A3H haptotypes II and V in order to determine whether there are shared biochemical characteristics between single Z-domain and double Z-domain A3 enzymes for restriction of HIV. The data demonstrate that A3H is a processive enzyme. A3H haptotype II uses sliding, jumping, and intersegmental transfer to locate deamination motifs on ssDNA. The Glu-178 polymorphism of A3H haptotype V causes diminished sliding and intersegmental transfer but does not affect jumping. This difference in processivity did not affect HIV restriction ability. Further, we identified that the dimerization interface in A3H is mediated by the pairing of β2 strands from two monomers, and tetramerization can occur through the pairing of loop 7 from two dimers, as predicted could occur from a crystal structure of APOBEC2 (A2) with a 40-amino acid N-terminal deletion (45). The β2-β2 strand dimerization of A3H is required for efficient ssDNA scanning. The results demonstrate that A3H dimerization and Asp-178 is required for sliding and intersegmental transfer movements on ssDNA. The data combine to support a model in which A3H haptotypes II and V both induce HIV mutagenesis efficiently but by different mechanisms and suggest that jumping can compensate for deficiencies in intersegmental transfer.

Of the A3 enzymes most effective against HIV, A3H is of special interest because it occurs with the highest number of haplotypes in the A3 family (13, 35, 36). A3H exists as at least seven haplotypes in humans, with three of the haplotypes being stable forms of A3H that can suppress HIV (Table 1). The A3H haplotypes that are unstable and inactive against HIV contain two single nucleotide polymorphisms that occur in different combinations with other polymorphisms. Unstable A3H haplotypes (haplotypes I, III, IV, and VI) carry either a Gly-105, ΔAsn-15, or both in combination with other polymorphisms at positions 18, 121, and 178 (35, 36) (Table 1). Unstable A3H haplotype I occurs at the highest population frequency (0.308 – 0.526) (35, 36). An Arg-105 and Asn-15 are required for a stable A3H in cells (35). The three stable haplotypes all have an Asn-15, Arg-18, and Arg-105 and vary at the 121 and 178 positions (35, 36) (Table 1). A3H haptotype II has been the most widely studied and has a ND sequence at positions 121 and 178 (37 – 39). A3H haptotype V has a DE sequence at positions 121 and 178 (13, 36). A3H haptotype II and V comprise the majority of the stable A3H alleles (haptotype II, frequency of 0.061 – 0.265; haptotype V, frequency of 0.054 – 0.202) (35, 36). A3H haptotype VII has been identified only from one individual (frequency of 0.009) and has a KE sequence at positions 121 and 178 (36). The 121 position determines the sensitivity to Vif, where an Asp-121 is sensitive and a Lys-121 is not sensitive to Vif-mediated degradation (13, 40, 41). There is currently no
tag. For generation of RNA in vitro, the HIV 5′-UTR (nucleotides 1–497) and human Alu sequence (46) were cloned into pSP72 vector (Promega) using XhoI and HindIII sites under the control of T7 promoter (5′-UTR) or SP6 promoter (Alu). All constructed plasmids were verified by DNA sequencing. Primers were obtained from Integrated DNA Technologies and are listed in supplemental Table S1.

**Protein Expression and Purification**—The pFASTbac1-GST-A3H vectors were used to produce recombinant baculovirus according to the protocol for the Bac-to-Bac system (Life Technologies). Recombinant GST-A3H baculovirus were then used to infect Sf9 cells at a multiplicity of infection of 20, and cells were harvested after 72 h. Cells were lysed, and clarified lysates were incubated with glutathione-Sepharose 4B resin (GE Healthcare) at 4 °C and subjected to a series of salt washes as described previously (47). For all A3H haplotypes and mutants except for the A3H haplotype II R44A/Y46A, D178K, and D178A mutants, on-column cleavage from the GST tag with thrombin (GE Healthcare) was performed at 21 °C for 18 h in thrombin digestion buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, and 1 mM DTT). Enzymes were assessed to be 95% pure by SDS-PAGE. Due to the lower expression of the A3H haplotype II R44A/Y46A, D178K, and D178A mutants, the enzyme was eluted with the GST tag in elution buffer (100 mM Tris, pH 8.8, 150 mM NaCl, 10% (v/v) glycerol, and 50 mM reduced glutathione). A wild type GST-haplotype II was purified in parallel with the mutant for comparative analyses. Eluted GST-A3H was dialyzed against 100 mM Tris, pH 7.5, 250 mM NaCl, 10% glycerol, and 1 mM DTT overnight at 4 °C. For size exclusion chromatography with the A3H haplotype II R44A/Y46A mutant, the GST tag was not cleaved to increase protein stability. For deamination reactions with the A3H haplotype II R44A/Y46A, D178K, and D178A mutants, the GST tag was cleaved in solution with thrombin (GE Healthcare).

**Single-cycle Infectivity Assay**—VSV-G pseudotyped HIV pNL4−3 ΔVif viruses were produced by transfecting 3 × 10⁵ 293T cells (ATCC CRL-3216) per well in a 6-well plate with GeneJuice (Novagen). Specifically, transfections used 500 ng of pHIVΔVif, which expresses an enhanced GFP reporter gene (48) and 200 ng of pMDG (49, 50), which expresses the VSV-G protein, in the presence or absence of A3H haplotype II or A3H haplotype V in pcDNA3.1. Empty pcDNA3.1 was used to achieve equivalent amounts of transfected DNA. A titration of A3H expression vector was used that ranged from 25 to 200 ng. The amino acid sequences of the A3s were identical to those used in biochemical assays. Twenty-four hours after the transfection, the medium was replaced. Virus-containing supernatants were collected 48 h after the medium change and filtered through 0.45-μm syringe filters. Virus was quantified by a p24 enzyme-linked immunosorbent assay (QuickTiter lentivirus titer kit (Cell Biolabs Inc.) or HIV-1 p24 ELISA kit (XpressBio)). Target 293T cells were infected at a multiplicity of infection of 0.5 by spinoculation at 800 × g for 1 h in the presence of 8 μg/ml Polybrene. Infection levels in 293T cells were determined by flow cytometry by detecting enhanced GFP fluorescence at 40 h postinfection, and data were normalized to HIVΔvif infections in the absence of A3 enzymes.

**Immunoblotting**—To detect A3H protein levels, HA-tagged versions of the A3H enzymes used for the single-cycle infectivity assays were constructed in pcDNA3.1. For detection of A3H in cell lysates (50 μg of total protein) and virions (50 ng of p24), anti-rabbit HA (Sigma) was used. Loading controls for cell lysates (α-tubulin, Sigma) and virions (p24, catalog no. 3537, National Institutes of Health AIDS Reagent Program) were detected using mouse monoclonal antibodies. Tagged proteins and loading controls were detected simultaneously on the same immunoblot by using the LI-COR/Odyssey system (IRDye 680-labeled goat anti-rabbit and IRDye 800-labeled goat anti-mouse secondary antibodies). Antibodies were used at a dilution of 1:1000.

**Sequence of Integrated Proviral DNA**—Infected 293T cells were harvested after 40 h, and the DNA was extracted using DNAzol reagent (Life Technologies). DNA was treated with DpnI (New England Biolabs) for 1 h at 37 °C to remove possible contaminating plasmid DNA, and the protease (prot) (nt 2280–2631) sequences were amplified by PCR using Q5 polymerase (New England Biolabs). Primers were obtained from Integrated DNA Technologies and are listed in supplemental Table S1. PCR products were purified and cloned with the Zero Blunt TOPO PCR cloning kit (Life Technologies) or New England Biolabs PCR cloning kit. DNA was sequenced with kit-specific primers and carried out at the National Research Council of Canada (Saskatoon, Canada) or Eurofins Genomics (Huntsville, AL).

**In Vitro Deamination Assay**—All ssDNA substrates were obtained from Tri-Link Biotechnologies and are listed in supplemental Table S1. All reactions were carried out under single-hit conditions (i.e., <15% substrate usage) to ensure that deaminations on each ssDNA were catalyzed by a single enzyme (51). Under these conditions, a processivity factor can be determined by comparing the total amount of deaminations occurring at two sites on the same ssDNA with a calculated theoretical value of deaminations at these two sites if the deamination events were uncorrelated (not processive) (28). In order to achieve <15% substrate usage, the ssDNA substrate containing two 5′-CTC motifs (100 nM) was incubated with 50 nM A3H for 1.5 to 20 min at 37 °C in deamination buffer containing 50 mM Tris, pH 7.5, 40 mM KCl, 10 mM MgCl₂, and 1 mM DTT. The reaction time was varied according to the specific activity of the enzyme on each ssDNA to achieve ~10% substrate usage. DNA substrates with a dsDNA or RNA/DNA hybrid were formed by heat annealing in 50 mM Tris, pH 7.5, and 50 mM NaCl. Reactions were started by the addition of the ssDNA substrate. To test whether intersegmental transfer was occurring, the A3H/ssDNA ratio (1:2) was kept constant, but increased amounts of enzyme and substrate were titrated into the reaction (ssDNA, 100–700 nM; A3H, 50–350 nM). A3H-catalyzed deaminations were detected by treating the ssDNA with uracil DNA glycosylase (New England Biolabs) and heating under alkaline conditions before resolving the fluorescein-labeled ssDNA on 10, 16, or 20% (v/v) denaturing polyacrylamide gels, depending on the sizes of the ssDNA fragments. Gel photos were obtained using a Typhoon Trio multipurpose scanner (GE Healthcare), and integrated gel band intensities were analyzed using ImageQuant (GE Healthcare) as described previously (28).
Size Exclusion Chromatography—The oligomerization states of A3H haplotype II, haplotype V, and haplotype II Y112A/Y113A were determined by loading 150 or 300 μg of the purified enzymes onto a Superdex 200 10/300 Increase column (GE Healthcare). The running buffer contained 20 mM Tris, pH 8.0, 300 mM NaCl, 10% (v/v) glycerol, and 1 mM DTT. The oligomerization states of GST-A3H haplotype II and GST-A3H haplotype II R44A/Y46A were examined by loading 10 μg of the purified enzymes onto a size exclusion chromatography column. The column was prepared by pouring a 10-ml Superdex 200 (GE Healthcare) resin bed contained in a column with a 0.5-cm diameter and 16-cm height. The running buffer contained 20 mM Tris, pH 8.0, 300 mM NaCl, 10% (v/v) glycerol, and 5 mM DTT. The Bio-Rad gel filtration standard set was used to generate a calibration curve from which the apparent molecular masses and oligomerization states of the enzymes were determined.

Steady-state Rotational Anisotropy—The apparent $K_d$ values of A3G, A3H haplotype II, and A3H haplotype V for ssDNA, dsDNA, RNA/DNA hybrid, or RNA were determined using steady state fluorescence depolarization (rotational anisotropy). The ssDNA substrate was the 118-nt ssDNA used for deamination assays and contained an internal fluorescein-dT (supplemental Table S1). The dsDNA and RNA/DNA hybrid were composed of 20 nt that matched the hybrid regions used to block A3H sliding in deamination assays (supplemental Table S1).

Fluorescently labeled RNA was produced by transcribing linearized pSP72 DNA (cut with EcoRI (5'-UTR) or HindIII (Alu)) in vitro using either T7 RNA polymerase (5'-UTR, Roche Applied Science) or SP6 polymerase (Alu, Roche Applied Science) with a nucleotide mixture containing fluorescein-dT (Roche Applied Science).

Reactions (50 μl) were conducted in deamination buffer and contained 50 nm fluorescein-labeled DNA or RNA and increasing amounts of A3 (A3G, 0–1000 nM; A3H haplotypes II and V, 0–1500 nM). A QuantaMaster QM-4 spectrofluorometer (Photon Technology International) with a dual emission channel was used to collect data and calculate anisotropy. Measurements were performed at 21°C. Samples were excited with vertically polarized light at 495 nm (6-nm band pass), and vertical and horizontal emissions were measured at 520 nm (6-nm band pass). The $K_d$ was obtained by fitting to a rectangular hyperbola or sigmoidal curve equation using SigmaPlot version 11.2 software.

Results

A3H Haplotype II and V Processively Scan ssDNA—The ssDNA scanning mechanism(s) of A3H haplotypes II and V were determined using an in vitro deamination assay that measures the processivity of purified A3H. Processivity is defined as the ability of an enzyme to deaminate more than one cytosine on an ssDNA substrate in a single enzyme-substrate encounter (28). The processivity was measured using different synthetic ssDNA substrates containing two deamination motifs (5'-CTC, where the underlined C is preferentially deaminated) spaced various distances apart. This strategy allows the dissection between one-dimensional sliding and three-dimensional translocation movements because it has been previously shown that substrates with closely spaced motifs (i.e., <20 nt) are processively deaminated through one-dimensional sliding motions (25, 26). Processive deamination of more distantly spaced motifs requires a three-dimensional translocation mechanism, such as jumping or intersegmental transfer (2, 25, 26). All deamination reactions were performed under “single-hit” conditions (<15% substrate usage) to ensure that any given ssDNA substrate was acted upon by at most one enzyme during the reaction (51). Under these conditions, a processivity factor can be calculated to determine the likelihood that an enzyme would undergo processive deaminations in comparison with a nonprocessive deamination (see “Experimental Procedures” and Ref. 28).

On ssDNA substrates with deamination motifs separated by 5 and 14 nt, A3H haplotype II had processivity factors of 2.1 and 3.6, respectively (Fig. 1, A and B). Thus, depending on the distance between the deamination motifs, A3H haplotype II is ~2–4 times more likely to catalyze a processive deamination of the two deamination motifs than a single nonprocessive deamination of one of the deamination motifs. Due to the short distance between the deamination motifs, the data show that A3H haplotype II can processively scan the ssDNA by sliding. The amino acid sequences of A3H haplotype II and A3H haplotype V differ only at the amino acid at position 178, where A3H haplotype II has an Asp and A3H haplotype V has a Glu (13, 36) (Table 1). Because the amino acid at position 178 is located on predicted helix 6, which mediates sliding ability in A3G and A3F (25, 26), we tested A3H haplotype V on ssDNA substrates with closely spaced deamination motifs to determine whether the sliding ability was compromised. On the substrate with deamination motifs separated by 5 nt, the processivity factor was 1 (Fig. 1A). Because the processivity factor is a ratio, the processivity factor of 1 means that A3H haplotype V is essentially not processive on this substrate (see “Experimental Procedures”), in contrast to A3H haplotype II (Fig. 1A). On the substrate with cytosines separated by 14 nt, A3H haplotype V is processive but has a processivity factor ~2-fold less than A3H haplotype II (compare processivity factors of 3.6 and 2.1 (Fig. 1B)). The data indicated that in A3H, predicted helix 6 mediates sliding and that the Asp/Glu-178 polymorphism between A3H haplotype II and haplotype V caused a difference in the ssDNA sliding ability.

We also tested whether other processive mechanisms were affected by the polymorphism at amino acid position 178. On the ssDNA substrate with cytosines separated by 63 nt, A3H haplotype V had a processivity factor of 3.8, which is ~1.5-fold less processive as compared with A3H haplotype II on the same substrate (compare processivity factors of 5.2 and 3.8 (Fig. 1C)). That both A3H haplotypes were processive on a substrate with distantly spaced deamination motifs suggests that A3H uses a three-dimensional translocation mechanism to scan ssDNA. To confirm this, we annealed a 20-nt complementary DNA between the two deamination motifs (Fig. 1D, schematic). Because A3H haplotype II does not bind to this dsDNA region (Table 2, no binding detected), it serves as a block to inhibit the sliding motion of the enzyme (28). A3H haplotype II can still processively deaminate the cytosines on this substrate (Fig. 1D,
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processivity factor of 2.2), which confirms that A3H haplotype II uses a three-dimensional translocation mechanism to overcome obstacles while scanning ssDNA. The ~2.5-fold decrease in processivity in comparison with the completely ssDNA

TABLE 1
A3H haplotypes

| Haplotype | Stability | Polymorphisms (amino acids 15, 18, 105, 121, and 178) |
|-----------|-----------|------------------------------------------------------|
| I         | Unstable  | NRGKE                                                |
| II        | Stable    | NRRDD                                                |
| III       | Unstable  | ΔRRDE                                                |
| IV        | Unstable  | ΔLDE                                                 |
| V         | Stable    | NRRR                                                 |
| VI        | Unstable  | ΔLGKE                                                |
| VII       | Stable    | NRRKE                                                |

TABLE 2
Comparison of apparent dissociation constants ($K_d$) from DNA of A3H haplotypes II, V, and A3G

| Enzyme         | ssDNA $K_d$ ± S.D. | dsDNA $K_d$ ± S.D. | RNA/DNA $K_d$ ± S.D. |
|----------------|---------------------|--------------------|----------------------|
| A3H haplotype II | 0.72 ± 0.14         | No binding detected | 0.65 ± 0.09          |
| A3H haplotype V  | 0.73 ± 0.07         | 7.30 ± 2.30        | 0.82 ± 0.29          |
| A3G             | 0.31 ± 0.04         | 5.70 ± 1.10        | 0.99 ± 0.11          |

FIGURE 1. Analysis of A3H haplotype II and V processivity and ssDNA scanning. Processivity of A3H haplotype II (Hap II) and haplotype V (Hap V) was tested on ssDNA substrates that contained fluorescein-labeled deoxythymidine (yellow star) between two 5’-CTC deamination motifs separated by different distances. A, deamination of a 60-nt ssDNA substrate with deamination motifs spaced 5 nt apart. Single deaminations of the 5’-C and 3’-C are detected as the appearance of labeled 42- and 23-nt fragments, respectively; double deamination of both C residues on the same molecule results in a 5-nt labeled fragment. B, deamination of a 69-nt ssDNA substrate with deamination motifs spaced 14 nt apart. Single deaminations of the 5’-C and 3’-C are detected as the appearance of labeled 51- and 32-nt fragments, respectively; double deamination of both C residues on the same molecule results in a 14-nt labeled fragment. C, deamination of a 118-nt ssDNA substrate with deaminated cytosines spaced 63 nt apart. Single deaminations of the 5’-C and 3’-C are detected as the appearance of labeled 100- and 81-nt fragments, respectively; double deamination of both C residues on the same molecule results in a 63-nt labeled fragment. D, deamination of the same substrate described for C but with a 20-nt DNA annealed between the two deamination motifs. E, deamination of the same substrate described for C but with a 20-nt RNA annealed between the two deamination motifs. A–E, the A3H/ssDNA ratio was 1:2. The measurements of enzyme processivity (processivity factor) and the S.D. are shown below the gels. All values are calculated from at least three independent experiments.
the dsDNA. (Table 2, apparent $K_d$ of 7.3 $\mu M$ (dsDNA) and 0.73 $\mu M$ (ssDNA)). Similar to A3H haplotype II, the dsDNA region caused a decrease in the processivity of A3H haplotype V, but the enzyme was still processive (Fig. 1D, processivity factor of 1.7). Interestingly, we found that A3H haplotypes II and V are able to bind RNA/DNA hybrids with an apparent dissociation constant that is equivalent to that of ssDNA (Table 2, A3H haplotype II apparent $K_d$ values of 0.65 $\mu M$ (RNA/DNA) and 0.72 $\mu M$ (ssDNA); A3H haplotype V apparent $K_d$ values of 0.82 $\mu M$ (RNA/DNA) and 0.73 $\mu M$ (ssDNA)). This is in contrast to A3G, which binds both dsDNA and RNA/DNA hybrids at least 3-fold less than ssDNA (Table 2, apparent $K_d$ values of 5.70 $\mu M$ (dsDNA), 0.99 $\mu M$ (RNA/DNA), and 0.31 $\mu M$ (ssDNA)). These data predict that A3H should be able to slide over RNA/DNA hybrids. To test this prediction, we annealed a 20-nt complementary RNA between the two deamination motifs (Fig. 1E, schematic). The enzyme processivity was not significantly different on this substrate in comparison with its analogous ssDNA for both A3H haplotypes II and V (Fig. 1, C and E, processivity factors). This suggests that during HIV reverse transcription, A3H haplotypes II and V could slide on RNA/DNA hybrid regions.

To determine the three-dimensional translocation mechanism of A3H haplotypes II and V, we tested whether the enzyme could move by intersegmental transfer. This mechanism requires that an enzyme with two DNA binding domains transfer to distal sites through a “doubly bound” intermediate state (32). A3H haplotype II has been shown to form dimers in solution (43), indicating that it could use intersegmental transfer. To observe intersegmental transfer, we increased the enzyme and substrate concentrations in the reaction but kept their ratios constant (Fig. 2). By crowding the reaction in this manner, the enzyme will become more likely to translocate to a different substrate than to translocate within a single ssDNA as the concentration of the reaction components increases (52). Thus, if intersegmental transfer is occurring, this would lead to an apparent decrease in the processivity factor. Intersegmental transfer should also result in an increase in the apparent reaction rate because increasing the cycling of the enzyme also increases the rate of searching for deamination motifs (52). The processivity factor of A3H haplotype II decreased ~2-fold with increasing reaction components (Fig. 2A, processivity factor decreases from 5.2 to 3.0), and this was accompanied by a ~2-fold increase in the rate of deamination (Fig. 2A, rate increases from 0.35 to 0.60%/min), demonstrating that A3H haplotype II can use intersegmental transfer to scan ssDNA. However, at the highest level of reaction components, the processivity plateaued and did not decrease to 1, which would otherwise indicate a complete loss of apparent processivity due to the reaction conditions (Fig. 2A, processivity factor of 3.0). This indicates that A3H haplotype II may also use jumping to translocate on ssDNA. Processive ssDNA scanning mediated by jumping is insensitive to the crowding of the reaction components because during this movement, the enzyme remains within the negatively charged region of the ssDNA and out of the bulk solution (2). Altogether, the results indicate that A3H haplotype II uses sliding and both intersegmental transfer and jumping as a three-dimensional translocation mechanism to scan ssDNA for its preferred deamination motif. We next examined whether A3H haplotype V was capable of intersegmental transfer. A3H haplotype V did have a 1.5-fold decrease in apparent processivity, similar to A3H haplotype II, but there was no increase in the reaction rate observed (Fig. 2, compare A and B). Thus, the data indicated that although A3H haplotype V could transfer to other ssDNA substrates, there was no corresponding increase in cycling between the ssDNAs, suggesting that the Glu-178 polymorphism also diminished intersegmental transfer ability. The processivity factor from the intersegmental transfer assay at the highest concentration of reaction components where the decrease in processivity plateaued was greater than 1, suggesting that A3H haplotype V was able to processively jump on ssDNA (Fig. 2B).

Of note, the identity of the amino acid at position 178 also affected the binding of dsDNA and RNA. Although A3H haplotypes II and V did not differ in their ssDNA binding ability as determined by their apparent dissociation constants ($K_d$) (Table 2, haplotype II $K_d$ of 0.72 $\mu M$; haplotype V $K_d$ of 0.73 $\mu M$), A3H haplotype V was able to bind dsDNA, whereas A3H haplotype II was not able to (Table 2, haplotype II, no binding...
TABLE 3

Comparison of apparent dissociation constants (K_d) and Hill coefficients from RNA of A3H haplotypes II and V

| Enzyme               | HIV 5′-UTR (Hill coefficient) | Alu (Hill coefficient) |
|----------------------|--------------------------------|------------------------|
| A3H haplotype II     | 0.22 ± 0.04 (2.1)              | 0.33 ± 0.05 (3.2)      |
| A3H haplotype V      | 4.60 ± 0.38 (1.8)              | 3.00 ± 0.60 (2.3)      |

detected; haplotype V, K_d of 7.3 μM). There were also haplotype differences in RNA binding. The apparent K_d of A3H haplotype II binding to HIV 5′-UTR RNA was 21-fold lower than A3H haplotype V (Table 3, haplotype II K_d of 0.22 μM; haplotype V K_d of 4.60 μM). This difference in RNA binding ability was consistent with binding data for 7SL. Alu RNA, where A3H haplotype II bound with an apparent K_d 9-fold lower than A3H haplotype V (Table 3, haplotype II K_d of 0.33 μM; haplotype V K_d of 3.00 μM). Interaction with either HIV or host RNAs, such as 7SL, in a nucleocapsid-dependent manner has been reported to be required for encapsidation of A3G and A3H (36, 53–60). Similar to A3G (46) and in agreement with previous data (44), A3H bound RNA cooperatively, demonstrating the ability to oligomerize on RNA (Table 3, Hill coefficients).

A3H Haplotypes II and V Demonstrate Similar HIVΔVif Restriction Efficiencies—Human A3H is the only single Z-domain A3 enzyme that encapsidates into budding HIV virions and restricts the replication of HIV in the subsequent target cell (3). It has been established that A3H haplotype II and haplotype V can similarly restrict the replication of HIV in cell culture or infected individuals (13, 35, 37, 39, 61–63). In agreement with others, our data indicated that in a single cycle of replication, A3H haplotype II and V suppress HIV similarly over a range of A3H expression levels (36, 39, 64, 65) (Fig. 3, A and B). Despite the 10–20-fold differences in RNA binding affinities between A3H haplotypes II and V (Table 3), their virion encapsidation was comparable (Fig. 3B). It has been shown that A3G binds cellular or viral RNA indiscriminately to ensure encapsidation into virions in a nucleocapsid-specific manner (66), and our data suggest that the same encapsidation mechanism applies to A3H.

It has been previously established for A3G and A3F that an efficient processive DNA scanning mechanism involving both one-dimensional sliding and three-dimensional translocation is required for inducing high numbers of deoxycytidine deaminations in HIV (−)-DNA (25). Because we observed that A3H haplotypes II and V were encapsidated into virions at similar levels to each other, and there were no significant differences in suppression of HIV infectivity (Fig. 3, A and B), the data indicate that the diminished processivity of A3H haplotype V in comparison with A3H haplotype II (Fig. 1) is consequential to A3H-mediated HIV restriction. This may be because A3H haplotype V did not have a complete loss of sliding ability and still maintained jumping as a three-dimensional translocation mechanism (Fig. 1). Further, the A3H haplotype II data suggest that the three-dimensional translocation mechanisms are redundant because A3H haplotype II did not restrict HIV replication more than A3H haplotype V over a range of cellular expression levels (Fig. 3, A and B). We sequenced proviral DNA from the transfection conditions that used 100 ng of A3H expression plasmid and found no significant differences in the spacing of the A3H haplotype II and V mutations, confirming that the diminished processivity of A3H haplotype V did not affect its ability to induce mutations in HIV proviral DNA (Fig. 3C). Processivity can be inferred from the mutational spectra because clustered mutations are indicative of sliding, and distantly spaced mutations are indicative of jumping or intersegmental transfer (25, 26, 67). Because there were similar distantly spaced mutations for both A3H haplotypes, the data further indicate that intersegmental transfer and jumping are redundant three-dimensional processive mechanisms (Fig. 3C). Both A3H haplotypes II and V also induced closely spaced mutations, indicative of sliding, in agreement with the processivity data from ssDNA oligonucleotides (Figs. 3C and 1 A and B). The 2-fold lower processivity of A3H haplotype V in comparison with A3H haplotype II for closely spaced deamination motifs on ssDNA oligonucleotide substrates (Fig. 1, A and B) did not appear to affect its ability to induce closely spaced mutations during proviral DNA synthesis (Fig. 3C). At most, there was only a 2-fold difference in the G to A mutation frequency of A3H haplotypes II and V, and this was observed only at the 100-ng A3H expression plasmid condition (Fig. 3A and Table 4).

Optimal Sliding and Intersegmental Transfer Require an Asp-178—The A3H haplotypes II and V differ by only an Asp/Glu at amino acid position 178. These amino acids have differences in side chain rigidity and pKa that could account for the effect on ssDNA scanning ability. However, we investigated whether a charge reversal or removal mutation could more drastically alter the processivity of A3H. The sliding ability of a D178K mutant was tested using an ssDNA substrate that had deamination motifs separated by 5 nt (Fig. 4A). Similar to A3H haplotype V, the A3H D178K mutant was not processive on this substrate, indicating a deficiency in sliding ability (compare Figs. 4A and 4B). In the assay that tests intersegmental transfer ability, the D178K mutant processivity decreased 1.6-fold with increasing concentration of enzyme and substrate, but there was no increase in the reaction rate (Fig. 4B). Thus, the D178K mutant was similar to A3H haplotype V and could transfer to other ssDNA substrates but was unable to cycle efficiently (compare Figs. 2B and 4B). Altogether, the data indicated that rather than the amino acid charge, the Asp at amino acid position 178 was specifically important for sliding and intersegmental transfer. However, a charged amino acid is important at amino acid position 178 for enzyme stability because a D178A mutant was less stable than A3H haplotype II and had processivity factors of 1.7 or less on all ssDNA substrates tested (data not shown). This suggests that a salt bridge may be formed from the amino acid position at 178 to stabilize the enzyme and influence interactions with ssDNA.

The Dimer Interface of A3H Is Mediated by Predicted β2-β2 Strand Interactions—The finding that an amino acid change on A3H predicted helix 6 affected sliding motions was consistent with published data (25, 26), but a role for helix 6 in interseg-
haplotype II and V both primarily form dimers in solution.

To fully characterize the determinants for intersegmental transfer, we characterized the oligomeric interfaces of A3H haplotype II in order to design mutations that would result in an A3H haplotype II monomer to determine the effect on intersegmental transfer in A3H, we characterized the oligomeric interfaces of remaining in its oligomeric state, and we had identified an amino acid determinant required for intersegmental transfer.

Because helix 6 did not mediate oligomerization for A3H (Fig. 5, A and B) as may have occurred based on A3C crystal structure contacts (68), we performed our structure-guided mutagenesis using another single Z-domain oligomerization model, the A2 crystal structure, and surrogate mutations made in activation-induced cytidine deaminase (45, 69). The 40-mer was used (32, 34). Additional determinants for this ssDNA scanning mechanism have not been previously identified. However, there appear to be additional determinants required for A3 enzymes because those with two Z-domains in a single polypeptide (i.e. A3F and A3G) are unable to readily undergo intersegmental transfer under our assay conditions (25). Based on intersegmental transfer requiring two DNA binding domains (32), the data indicated that because we could not completely abolish intersegmental transfer ability by making mutations at the amino acid at position 178, the A3H was remaining in its oligomeric state, and we had identified an amino acid determinant required for intersegmental transfer. To fully characterize the determinants for intersegmental transfer in A3H, we characterized the oligomeric interfaces of A3H haplotype II in order to design mutations that would result in an A3H haplotype II monomer to determine the effect on intersegmental transfer.

Using size exclusion chromatography, we observed that A3H haplotype II and V both primarily form dimers in solution (apparent molecular mass of 44 kDa; Fig. 5, A and B). We also observed minor monomer (apparent molecular mass of 24 kDa) and tetramer (apparent molecular mass of 98 kDa) peaks (Fig. 5, A and B). A dimer and tetramer of A3H are of equivalent molecular mass to a monomer and dimer of A3G, respectively (46).

The formation of A3H tetramers was not concentration-dependent because the chromatograms for 150 and 300 μg of enzyme showed that the tetramer fraction remained at only 20% (haplotype II) or 25% (haplotype V) of the total population (Fig. 5, A and inset graph). These data confirmed that Asp-178 was not responsible for mediating dimerization, and instead the amino acid at position 178 is a specific determinant that contributes to intersegmental transfer ability.

### Table 4

| Enzyme          | Base pairs sequenced | Total number of G→A mutations | G→A mutation frequency (mutations/kb) |
|-----------------|----------------------|-------------------------------|---------------------------------------|
| A3H haplotype II| 10,530               | 35                            | 3.2                                   |
| A3H haplotype V | 8424                 | 14                            | 1.7                                   |

FIGURE 3. A3H haplotypes II and V demonstrate similar HIVΔVif restriction efficiencies. A, HIVΔVif infectivity was measured by enhanced GFP expression in 293T cells infected with HIVΔVif that was produced in the absence or presence of untagged A3H haplotype II (Hap II) or A3H haplotype V (Hap V). A titration of A3H plasmid was used (25, 50, 100, or 200 ng). Results normalized to the no A3 condition are shown with the S.D. (error bars) calculated from at least three independent experiments. B, the level of HA-tagged A3H haplotype II and A3H haplotype V expressed in cells and encapsidated into HIVΔVif virions was determined using immunoblotting. The A3 enzymes were detected through the HA tag. The loading control for cell lysates was α-tubulin (α-tub), and the loading control for virions was p24. A representative blot from three independent experiments is shown. C, proviral DNA sequencing data from a subset of clones (from 100 ng of A3H plasmid transfection) showing the sequence context of mutations induced by haplotype II or V in the protease region. Asterisks denote homology. Guanine (G) to adenine (A) mutations are shown in boldface type.
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**FIGURE 4. A3H D178K is deficient in sliding and intersegment transfer.** Processivity of A3H D178K was tested on ssDNA substrates that contained fluorescein-labeled deoxothyridine (yellow star) between two 5'-CTC deamination motifs separated by different distances. A, deamination of a 60-nt ssDNA substrate with deamination motifs spaced 5 nt apart. Single deaminations of the 5'C and 3'C are detected as the appearance of labeled 42- and 23-nt fragments, respectively; double deamination of both C residues on the same molecule results in a 5-nt labeled fragment. For D178K, the 100- and 81-nt fragments, respectively; double deamination of both C residues on the same molecule results in a 63-nt labeled fragment. For D178K, the 100- and 81-nt fragments, respectively; double deamination of both C residues on the same molecule results in a 63-nt labeled fragment. For D178K, the 100- and 81-nt fragments, respectively; double deamination of both C residues on the same molecule results in a 63-nt labeled fragment. For D178K, the 100- and 81-nt fragments, respectively; double deamination of both C residues on the same molecule results in a 63-nt labeled fragment.

A3H (Fig. 5) was not cleaved to extend the stability of the mutant for size exclusion chromatography because the R44A/Y46A mutant was prone to precipitation after extended incubation at 4 °C in solution without GST. Only 10 μg of GST-A3H wild type and mutant were applied to the column due to the lower yield of A3H haplotype II R44A/Y46A mutant during purification, and the chromatograms were generated by manually quantifying integrated gel band intensities in each fraction from an SDS-polyacrylamide gel. Further, the GST-tagged wild type and mutants were analyzed on a 10-ml G200 Superdex column, in contrast to untagged A3H that was analyzed using a 25-ml G200 Superdex column (see “Experimental Procedures”). To ensure that the GST tag did not confound our interpretation of the results, the GST-tagged R44A/Y46A mutant was compared with GST-tagged A3H haplotype II during the size exclusion chromatography. Due to the 22-kDa GST tag, the predicted GST-A3H monomer molecular mass was 46 kDa. Consistent with β2-β2 strand dimerization, the GST-A3H haplotype II R44A/Y46A mutant eluted predominantly as a monomer (apparent molecular mass of 37 kDa; Fig. 5, D–F). Although GST alone forms a dimer (apparent molecular mass of 51 kDa; Fig. 5G), the GST appears to be unable to dimerize when attached to the A3H protein (Fig. 5D); otherwise, we would be unable to resolve GST-A3H haplotype II R44A/Y46A at a molecular mass of 37 kDa. GST-A3H haplotype II resolved as a dimer (apparent molecular mass of 77 kDa; Fig. 5, D–F), consistent with the untagged A3H haplotype II data (Fig. 5A). There is a clear peak shift of the GST-A3H haplotype II and GST-A3H haplotype II R44A/Y46A in the SDS-PAGE analysis of the fractions, suggesting that GST-A3H haplotype II R44A/Y46A fully disrupted dimerization (Fig. 5, D and F). Nonetheless, in an attempt to examine the extent of the β2 strand contacts and whether GST-A3H haplotype II R44A/Y46A mutant fully disrupted dimerization, we also made a GST-A3H haplotype II P42S mutant, but this mutant was not stably produced from the S9 cell system. These data support the conclusion that A3H dimerization is mediated by multiple contacts along the predicted β2 strand and involves at least amino acid residues R44RGY46. Further, these data demonstrated that the A3H haplotype II dimer was stable over a 15-fold concentration range (Fig. 5, compare A and D).

According to the A2 single Z-domain oligomerization model, a β2-β2 mediated dimer can tetramerize via hydrophobic amino acids on loop 7 (45). A3H haplotype II formed tetramers in solution (Fig. 5A). To investigate the amino acids involved in A3H tetramerization, we made an A3H haplotype II loop 7 double mutant, Y112A/Y113A (Fig. 6A). During size exclusion chromatography of 150 μg of A3H haplotype II Y112A/Y113A, predominantly dimers were formed (Fig. 6B, apparent molecular mass of 39 kDa), similar to wild type A3H haplotype II (Fig. 5A). In contrast to wild type A3H haplotype II, the Y112A/Y113A mutant formed fewer tetramers (Fig. 6B, peak denoted by T). At 300 μg of A3H haplotype II Y112A/Y113A, the mutant had no increase in tetramer formation, suggesting that Tyr-112 and Tyr-113 primarily contribute to tetramerization (Fig. 6B, inset graph). However, we could not test this hypothesis further because another loop 7 mutant, A3H haplotype II H114A/W115A, could not be stably produced from S9 cells, in agreement with the low cellular stability observed by others (36, 38). Altogether, the data indicate that A3H haplotype II forms dimers through β2 strand interactions and tetramerizes through loop 7.

In solution, the tetramers were a small proportion of the total A3H (Fig. 5A). However, A3H haplotype II bound ssDNA cooperatively (Fig. 6C, Hill coefficient of 1.8), suggesting that tetramerization was promoted by A3H dimers binding ssDNA. In support of this hypothesis, the A3H haplotype II Y112A/Y113A mutant bound ssDNA with an apparent Kd similar to that of A3H haplotype II, but the saturation curve fit a noncooperative rectangular hyperbola (Fig. 6C). Together, these data indicate the formation of tetramers on ssDNA. A3H haplotype II Y112A/Y113A was not enzymatically active on several substrates with different deamination motifs (e.g. 5’-ATC, 5’- TTC, 5’-CTC, 5’-GTC, 5’-CCC, and 5’-AAC, in agreement with a previous study (36, 38) and data not shown). Loop 7 is known to
interact with ssDNA and mediate deamination motif preference in DNA binding models for multiple APOBEC deaminases (49, 70–72). Because the A3H haplotype II Y112A/Y113A mutant bound ssDNA with an apparent $K_d$ similar to that of A3H haplotype II, the data indicated that the loop 7 mutant was probably inactive due to an altered conformation of ssDNA in the active site (Fig. 6C).

A3H haplotype II β2 Strand Mutant Exhibits Compromised Sliding and Intersegmental Transfer—We tested A3H haplotype II R44A/Y46A to determine whether this β2 strand mutant

FIGURE 5. β2-β2 strand amino acids mediate A3H dimerization. Size exclusion chromatography profiles of A3H haplotype II (Hap II) and A3H haplotype V (Hap V) obtained from a 25-ml G200 Superdex Increase column (A) were used to calculate the oligomerization states of the enzymes from a standard calibration curve (B). Analysis demonstrated that both A3H haplotype II and A3H haplotype V were able to form monomers (M), dimers (D), and tetramers (T) in solution. Both 150 and 300 µg (inset graph) of enzyme were resolved to investigate whether A3H tetramer formation was concentration-dependent. According to the calibration curve, the apparent molecular masses of peak fractions for A3H haplotype II were 24 kDa (monomer), 44 kDa (dimer), and 94 kDa (tetramer), and for A3H haplotype V, they were 23 kDa (monomer), 44 kDa (dimer), and 102 kDa (tetramer). Sequence alignment of A3H haplotype II and A2 β2 strand amino acid sequences. Amino acids that were mutated are indicated in red. The size exclusion chromatography profiles of GST-A3H haplotype II (GST-HapII) and GST-A3H haplotype II R44A/Y46A (GST-R44A/Y46A) obtained from a 10-ml G200 Superdex column (D) were used to calculate the oligomerization states of the enzymes from a standard calibration curve (E). When 10 µg of enzyme was loaded onto the size exclusion column, GST-A3H haplotype II formed dimers in solution (apparent molecular mass of 77 kDa in peak fraction). This is in contrast to GST-A3H haplotype II R44A/Y46A, which formed monomers (apparent molecular mass of 37 kDa in peak fraction). The chromatograms from the 10-ml Sephadex 200 column (D) were constructed by analyzing the integrated gel band intensities of the protein in each fraction after resolution by SDS-PAGE. The gels show the peak fractions of GST-A3H haplotype II and GST-A3H haplotype II R44A/Y46A with start and end volumes corresponding to the fractions that were resolved by SDS-PAGE. G, the Sephadex 200 column (10-ml bed volume) demonstrates that GST is a dimer. The gel shows the peak fractions of GST with start and end volumes corresponding to the fractions that were resolved by SDS-PAGE. However, GST-tagged A3H does not dimerize through the GST tag based on data from GST-A3H haplotype II R44A/Y46A (D). AU, absorbance units.
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A

Loop 7

Hap II

A2

109 SRLYYHWCKP-118

153 GRLF-MWEEPE-162

B

FIGURE 6. Loop 7 amino acids Tyr-112 and Tyr-113 mediate A3H tetramerization. A, sequence alignment of A3H haplotype II (Hap II) and A2 loop 7 amino acid sequences. Amino acids that were mutated are indicated in red. B, the size exclusion chromatography profile of A3H haplotype II Y112A/Y113A (Y112A/Y113A, 150 μg) was used to calculate the oligomerization state of the enzyme from a standard calibration curve (Fig. 5B). Haplotype II Y112A/Y113A formed dimers (apparent molecular mass of 39 kDa in peak fraction). Overlay, haplotype II wild type (dashed line) is shown from Fig. 5A for comparison. A3H haplotype II Y112A/Y113A has less of a tetramer peak than haplotype II wild type. Inset graph, A3H haplotype II Y112A/Y113A did not resolve with a tetramer peak even when 300 μg of enzyme was resolved on the size exclusion column. C, the apparent $K_d$ of A3H haplotype II and A3H haplotype II Y112A/Y113A from a 118-nt ssDNA was analyzed by steady-state rotational anisotropy. The A3H haplotype II bound ssDNA with a best least squares fit to a sigmoidal binding curve (Hill coefficient of 1.8) and an apparent $K_d$ of 0.72 ± 0.14 μM. The A3H haplotype II Y112A/Y113A mutant bound ssDNA with a best least squares fit to a rectangular hyperbola and an apparent $K_d$ of 0.70 ± 0.13 μM. AU, absorbance units. Error bars, S.D. from three independent experiments.

C

DNA bound (nM)

A3H (nM)

was still able to processively scan ssDNA. Interestingly, the A3H haplotype II R44A/Y46A mutant bound ssDNA with a cooperative binding curve (Fig. 7A, apparent $K_d$ of 0.21 μM; Hill coefficient of 1.6), suggesting that the mutant can dimerize through loop 7 amino acids on ssDNA and does not remain a monomer as in solution (Fig. 5D). Because the Y112A/Y113A mutant was not catalytically active (data not shown), we were unable to test the deamination ability of an A3H R44A/Y46A/Y112A/Y113A mutant. Nonetheless, testing the A3H haplotype II R44A/Y46A would reveal the function of the β2-β2 strand dimer on catalytic activity and ssDNA scanning. We found that the A3H haplotype II R44A/Y46A could still slide to processively deaminate multiple cytosines but not as efficiently as A3H haplotype II. On an ssDNA substrate with cytosine separated by 5 nt, the A3H haplotype II R44A/Y46A had a processivity factor of 1, demonstrating that it was essentially not able to processively deaminate closely spaced cytosines (Fig. 7B), in contrast to A3H haplotype II, which had a processivity factor of 2.1 on this substrate (Fig. 1A). When the deamination motifs were spaced further apart, but still within a distance that requires sliding for processive deamination, the A3H haplotype II R44A/Y46A mutant was able to processively deaminate the two motifs (Fig. 7C, deamination motifs 14 nt apart, processivity factor of 2.1), but the sliding was not as efficient as A3H haplotype II (Fig. 1B, processivity factor of 3.6). These data indicated that although the A3H haplotype II R44A/Y46A mutant had less of a helix 6 that was able to mediate sliding, the sliding movement was compromised in the absence of the β2-β2 strand dimer. The β2 strand dimerization may form a ssDNA binding groove that promotes processive sliding in combination with helix 6.

To examine whether A3H haplotype II R44A/Y46A was still capable of undergoing intersegmental transfer, we used the assay that was used for A3H haplotypes II and V (Fig. 2). The processivity of A3H haplotype II R44A/Y46A did show a 2-fold decrease in the apparent processivity with an increased concentration of the reaction components (Fig. 7D), similar to A3H haplotype II (Fig. 2A). However, the rate of the reaction did not increase as the reaction components increased (compare Figs. 2A and 7D). These data indicate that the A3H haplotype II R44A/Y46A could transfer to other ssDNA substrates, probably due to the dimerization mediated by loop 7 (Fig. 7A, cooperative binding curve), but did not readily cycle through the ssDNA substrates (Fig. 7D, no increase in reaction rate). This may be due to the loop 7 dimer being less stable than the β2 strand dimer, which is supported by the lack of a concentration dependence of tetramer formation in solution (Fig. 5A). However, the data suggested that A3H haplotype II R44A/Y46A mutant was able to undergo three-dimensional translocations because the processivity on the ssDNA substrate with the deamination motifs separated by 63 nt was similar to that of A3H haplotype II (compare processivity factors of 5.2 (Fig. 1C) and 4.6 (Fig. 7D, first reaction lane)). The three-dimensional translocation ability of A3H haplotype II R44A/Y46A was confirmed by demonstrating that the mutant retained processivity in the presence of a dsDNA region between the deamination motifs (Fig. 7E).

Discussion

Human A3H is the most polymorphic human A3 enzyme with seven different haplotypes having different cellular stabilities (13, 35, 36). In comparison with other A3s, stable A3H

Discussion

Human A3H is the most polymorphic human A3 enzyme with seven different haplotypes having different cellular stabilities (13, 35, 36). In comparison with other A3s,
haplotypes are most able to have a restrictive effect on the progression of an HIV infection in the early stages (37, 39, 61). This is due to A3H not being sensitive to all of the HIV Vif variants, unlike A3G and A3F (64, 73, 74). Because the majority of humans of non-African descent carry A3H alleles that code for an unstable form of A3H, not all HIV Vif variants are adapted to

FIGURE 7. Analysis of A3H haplotype II β2 strand mutant R44A/Y46A ssDNA binding and scanning. A, A3H haplotype II (Hap II) R44A/Y46A ssDNA binding was analyzed by steady-state rotational anisotropy. The apparent dissociation constant ($K_d$) of A3H haplotype II R44A/Y46A from the 118-nt ssDNA was $0.21 \pm 0.04 \mu M$. The binding curve had a best least squares fit to a sigmoidal binding curve (Hill coefficient 1.6), demonstrating that the mutant could still oligomerize on ssDNA. Error bars, S.D. B–E, processivity of A3H haplotype II R44A/Y46A (R44A/Y46A) was tested on ssDNA substrates that contained fluorescein-labeled deoxythymidine (yellow star) between two 5’-CTC deamination motifs separated by different distances. B, deamination of a 60-nt ssDNA substrate with deamination motifs spaced 5 nt apart. Single deaminations of the 5’-C and 3’-C are detected as the appearance of labeled 42- and 23-nt fragments, respectively; double deamination of both C residues on the same molecule results in a 5-nt labeled fragment. C, deamination of a 69-nt ssDNA substrate with deamination motifs spaced 14 nt apart. Single deaminations of the 5’-C and 3’-C are detected as the appearance of labeled 51- and 32-nt fragments, respectively; double deaminations of both C residues on the same molecule results in a 14-nt labeled fragment. D, deamination of a 118-nt ssDNA substrate with deamination motifs spaced 63 nt apart. Single deaminations of the 5’-C and 3’-C are detected as the appearance of labeled 100- and 81-nt fragments, respectively; double deamination of both C residues on the same molecule results in a 63-nt labeled fragment. For this assay, the R44A/Y46A/ssDNA ratio of 1:2 was kept constant, but the total reaction components were increased as 50 nM/100 nM, 100 nM/200 nM, 150 nM/300 nM, 200 nM/400 nM, 250 nM/500 nM. These reaction conditions enabled the investigation of whether A3H haplotype II R44A/Y46A could translocate on ssDNA by intersegment transfer. The reaction rate of deamination (percent/min) for each individual reaction condition is shown below the gel. E, deamination of the same substrate described for B, C, and D, but with a 20-nt DNA annealed between the two cytosines. For reactions performed on substrates described for B, C, and E, the A3H/ssDNA ratio was 1:2. The measurements of enzyme processivity (processivity factor) and the S.D. are shown below the gels. All values are calculated from at least three independent experiments. A white line between lanes in gel images indicates that an intervening lane(s) on the same gel was cropped for visualization.
induce degradation of A3H if they are circulating in a geographical location where the majority of the population carries unstable A3H alleles (35–37, 39). Thus, if an HIV strain from a human with inactive A3H alleles infects a human with a stable A3H allele, the Vif will have to adapt to degrade the stable A3H (37, 39). It has been found that stable A3H can suppress HIV replication and delay time to treatment early in infection (37). Thus, stable A3H alleles could potentially act as an HIV infection barrier (39).

Despite the recognized importance of A3H in the A3 repertoire that can suppress HIV replication, there has been no biochemical characterization of the ssDNA scanning mechanisms of A3H, a biochemical comparison of stable haplotypes, or identification of the residues mediating oligomerization. Because oligomerization is required for virion encapsidation and enables A3H to be the only single Z-domain A3 capable of restricting HIV replication (3, 44), understanding the interfaces where oligomerization occurs is important for realizing the determinants for HIV restriction. Thus, we focused our study on the two stable haplotypes, II and V, which occur with the highest frequency in humans (35, 36). Despite differing by only a single amino acid, we found biochemical differences in haplotypes II and V. The more rigid side chain and lower isoelectric point of the A3H haplotype II Asp-178 in comparison with the SNP of A3H haplotype II R44A/Y46A to ssDNA, signifying the formation of oligomers, our data show that intersegmental transfer ability can be mediated by A3H, a function of the A3H amino acid 178 polymorphism, and that helix 6 can influence dsDNA and RNA binding mechanisms and the binding affinity for dsDNA and RNA. Our study of A3H has established the basis of processivity for a single Z-domain A3 enzyme, that redundant processive mechanisms are used by A3H, a function of the A3H amino acid 178 polymorphism, and that helix 6 influences dsDNA and RNA binding affinity.

In general, an enzyme that moves processively on DNA using intersegmental transfer must have at least two DNA binding domains to mediate the double bound intermediate state where the enzyme simultaneously binds two segments of DNA before transferring to one of the bound segments (32, 34). However, our data show that intersegmental transfer ability can be mediated by A3H at different levels. Both A3H haplotype V and A3H haplotype II R44A/Y46A have altered intersegmental transfer ability but for different reasons (Figs. 2B and 2D). The A3H haplotype II and V data (Fig. 2) demonstrate that Asp-178 on either loop 7 mutant was not catalytically active, in agreement with previous studies (36, 38) (data not shown).

A3H haplotype II was found to use several processive mechanisms to scan ssDNA. A3H haplotype II could slide on ssDNA, slide over RNA/DNA hybrids, and translocate by intersegmental transfer and jumping (Figs. 1 and 2A). Although A3H haplotype V exhibited diminished sliding and intersegmental transfer ability (Figs. 1A and B) and 2B), the enzyme was able to jump (Figs. 1C and D) and restricted HIV replication similarly to A3H haplotype II (Fig. 3). Thus, the data indicate that for A3H, the three-dimensional translocation mechanisms of intersegmental transfer and jumping are redundant. A3H haplotype V could processively slide on ssDNA to deaminate cytosines that were 14 nt apart but not 5 nt apart (Fig. 1A and B). However, A3H haplotype V-mediated mutagenesis of HIV was not adversely affected, despite a lack of a redundant mechanism for searching for closely spaced cytosines (Fig. 3). These data suggest that deficiencies in sliding are not as detrimental to the efficiency of the search for deamination motifs as deficiencies in jumping. This is supported by A3G reaction conditions and A3G mutants that removed either sliding or jumping ability (26, 67). For A3G, larger decreases in mutagenic efficiency result from losses in jumping rather than sliding (26, 67). Altogether, the A3H data are consistent with our previous work, which found that a high A3 mutagenic potential during HIV reverse transcription requires the enzyme to use both a one-dimensional and three-dimensional translocation mechanism (25). Our data are also in agreement with the findings of others who have shown A3H haplotype V to be as restrictive as A3H haplotype II in primary cells during a spreading infection and in single-cycle infectivity assays (36, 39, 64, 65).

Our results indicate that A3H oligomerizes similarly to the model proposed for the N-terminal 40 amino acids-deleted A2 crystal structure (45). The A3H dimer is highly stable, and even at very low concentrations of A3H, the dimer interface was maintained (Fig. 5D, 10 μg). The A2 model of oligomerization where a single Z-domain enzyme dimerizes through the β2 strands and tetramerizes through loop 7 has been questioned...
because this structure only forms in the N-terminal deleted version of A2, and human A2 in vitro activity has yet to be demonstrated (44, 45, 77). The A3H data demonstrate that the A2 model of oligomerization is a valid model for single Z-domain enzymes. A caveat of our data is that we could not produce a β2 strand mutant that was as stable as wild type A3H haplotype II. Thus, we required a GST tag to stabilize the β2 strand mutant. It is known that GST alone forms dimers (Fig. 5G), but our data demonstrate that GST-A3H does not form dimers through the GST tag, or we would be unable to resolve A3H haplotype II R44A/Y46A as a monomer (Fig. 5D). These data suggest that the A3H can block GST dimerization in our GST-A3H fusion construct. Nonetheless, this model of oligomerization does not appear to be conserved across all Z-type domains because multiple crystal structures of single Z-domain enzymes or C-terminal domains of double Z-domain enzymes have not oligomerized according to the A2 model (68, 78 – 82). Because A3H is the only Z3-domain in humans, it may be the only A3 to oligomerize in this manner (42). A3H tetramers form primarily during DNA binding and not in solution. This is supported by the data for the A3H haplotype II β2 strand mutant R44A/Y46A that could still bind ssDNA cooperatively (Fig. 7A) and the A3H haplotype II loop 7 mutant Y112A/Y113A that could not (Fig. 6C). These data are also consistent with size exclusion chromatography profiles of A3H haplotypes II and V, where tetramers were a minority of the population in comparison with dimers (Fig. 5A). Mitra et al. (38) have investigated the role of loop 7 in the A3H-ssDNA interactions required for catalytic activity. Deamination reactions conducted with A3H haplotype II and mutants expressed from 293T cells demonstrated that Y112A and W115A mutants lose deamination activity (38). Interestingly, W115F rescued the deamination activity by 30%, suggesting that aromatic residues on loop 7 are involved in nucleic acid interactions that are required for catalysis through base stacking (38), in agreement with data from activation-induced cytidine deaminase (83). However, this does not affect the apparent $K_d$ from ssDNA (Fig. 6C).

The A3H dimer organization may provide an advantage by decreasing the Vif-mediated degradation efficiency and thus promoting the HIV restriction ability of A3H. We previously showed that A3G dimerization is disrupted by Vif (43). This may contribute to efficient Vif-induced polyubiquitination. Vif can disrupt A3G dimerization because the primary residue with which Vif interacts on A3G is Asp-128, which is adjacent to the primary dimerization amino acids, Phe-126 and Trp-127 (18, 43, 46, 84 – 86). In contrast, when A3H haplotype II interacted with Vif, there was a structural change induced by Vif, but this did not appear to be a complete disruption of dimerization (43). Our data on the A3H dimer interface suggest that this is because A3H dimerizes through the β2 strands (Fig. 5D) and Vif interacts with A3H on a separate structure, predicted helix 4 (87, 88). Using quantitative immunoblotting, it was shown that Vif$_{A3H}$ is 4-fold less efficient at degrading A3H than A3G. Further, both Vif$_{NL4-3}$ and Vif$_{HXB2}$ could induce polyubiquitination of A3H with Lys-63 and Lys-48 linkages, in contrast to A3G that was polyubiquitinated with only Lys-48 linkages (43). Thus, the A3H β2 strand dimer may decrease the effectiveness of Vif and promote alternate ubiquitination modifications.

Although during viral infections, HIV Vif can effectively suppress both A3G and A3H to enable viral replication (74), the higher resistance of A3H to Vif may provide an advantage during the early stages of infection and during Vif adaptation (37, 39).

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**References**

1. Desimnie, B. A., Delviks-Frankenberrry, K. A., Burdick, R. C., Qi, D., Izumi, T., and Pathak, V. K. (2014) Multiple APOBEC3 restriction factors for HIV-1 and one Vif to rule them all. *J. Mol. Biol.* 426, 1220 – 1245

2. Feng, Y., Baig, T. T., Love, R. P., and Chelico, L. (2014) Suppression of APOBEC3-mediated restriction of HIV-1 by Vif. *Front. Microbiol.* 5, 450

3. Hultquist, J. F., Lengyl, J. A., Refsland, E. W., LaRue, R. S., Lackey, L., Brown, W. L., and Harris, R. S. (2011) Human and rhesus APOBEC3D, APOBEC3F, APOBEC3G, and APOBEC3H demonstrate a conserved capacity to restrict Vif-deficient HIV-1. *J. Virol.* 85, 11220 – 11234

4. Refsland, E. W., Hultquist, J. F., and Harris, R. S. (2012) Endogenous origins of HIV-1 G-to-A hypermutation and restriction in the nonpermissive T cell line CEMzn. *PLoS Pathog.* 8, e1002800

5. Harris, R. S., Bishop, K. N., Sheehy, A. M., Craig, H. M., Petersen-Mahrt, S. K., Watt, I. N., Neuberger, M. S., and Malim, M. H. (2003) DNA deamination mediates innate immunity to retroviral infection. *Cell* 113, 803 – 809

6. Mangeat, B., Tirelli, P., Caron, G., Friedli, M., Perrin, L., and Trono, D. (2003) Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature* 424, 99 – 103

7. Zhang, H., Yang, B., Pomerantz, R. J., Zhang, C., Arunachalam, S. C., and Gao, L. (2003) The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. *Nature* 424, 94 – 98

8. Yu, Q., König, R., Pillai, S., Chiles, K., Kearney, M., Palmer, S., Richman, D., Coffin, J. M., and Landau, N. R. (2004) Single-strand specificity of APOBEC3G accounts for minus-strand deamination of the HIV genome. *Nat. Struct. Mol. Biol.* 11, 435 – 442

9. Liddament, M. T., Brown, W. L., Schumacher, A. J., and Harris, R. S. (2004) APOBEC3F properties and hypermutation preferences indicate activity against HIV-1 in vivo. *Curr. Biol.* 14, 1385 – 1391

10. Wiegand, H. L., Doehle, B. P., Bogerd, H. P., and Cullen, B. R. (2004) A second human antiretroviral factor, APOBEC3F, is suppressed by the HIV-1 and HIV-2 Vif proteins. *EMBO J.* 23, 2451 – 2458

11. Zheng, Y. H., Irwin, D., Kurusu, T., Tokunaga, K., Sata, T., and Peterlin, B. M. (2004) Human APOBEC3F is another host factor that blocks human immunodeficiency virus type 1 replication. *J. Virol.* 78, 6073 – 6076

12. Dang, Y., Wang, X., Esselman, W. J., and Zheng, Y. H. (2006) Identification of APOBEC3DE as another antiretroviral factor from the human APOBEC family. *J. Virol.* 80, 10522 – 10533

13. Harari, A., Ooms, M., Mulder, L. C., and Simon, V. (2009) Polymorphisms and splice variants influence the antiretroviral activity of human APOBEC3H. *J. Virol.* 83, 295 – 303

14. Harris, R. S., Hultquist, J. F., and Evans, D. T. (2012) The restriction factors of human immunodeficiency viruses. *J. Biol. Chem.* 287, 40875 – 40883

15. Sheehy, A. M., Gaddis, N. C., Choi, J. D., and Malim, M. H. (2002) Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* 418, 466 – 4650

16. Conticello, S. G., Harris, R. S., and Neuberger, M. S. (2003) The Vif protein of HIV triggers degradation of the human antiretroviral DNA deaminase APOBEC3G. *Curr. Biol.* 13, 2009 – 2013
39. Relfsland, E. W., Hultquist, J. F., Luengas, E. M., Ikeda, T., Shaban, N. M., Law, E. K., Brown, W. L., Reilly, C., Emerman, M., and Harris, R. S. (2014) Natural polymorphisms in human APOBEC3H and HIV-1 Vif combine in primary T lymphocytes to affect viral G-to-A mutation levels and infectivity. PLoS Genet. 10, e1004761

40. Zhen, A., Wang, T., Zhao, K., Xiong, Y., and Xu, Y. F. (2010) A single amino acid difference in human APOBEC3 variants determines HIV-1 Vif sensitivity. J. Virol. 84, 1902–1911

41. Li, M. M., and Emerman, M. (2011) Polymorphism in human APOBEC3H affects a phenotype dominant for subcellular localization and antiviral activity. J. Virol. 85, 8197–8207

42. LaRue, R. S., Jonsson, S. R., Silverstein, K. A., Lajoie, M., Bertrand, D., El-Mabrouk, N., Hétzel, I., Andresdottir, V., Smith, T. P., and Harris, R. S. (2008) The artiodactyl APOBEC3 innate immune repertoire shows evidence for a multi-functional domain organization that existed in the ancestor of placental mammals. BMC Mol. Biol. 9, 104

43. Baig, T. T., Feng, Y., and Chelico, L. (2014) Determinants of efficient degradation of APOBEC3 restriction factors by HIV-1 Vif. J. Virol. 88, 14380–14395

44. Li, J., Chen, Y., Li, M., Carpenter, M. A., McDougle, R. M., Luengas, E. M., Macdonald, P. J., Harris, R. S., and Mueller, J. D. (2014) APOBEC3 multimerization correlates with HIV-1 packaging and restriction activity in living cells. J. Mol. Biol. 426, 1296–1307

45. Prochnow, C., Bransteitter, R., Klein, M. G., Goodman, M. F., and Chen, X. S. (2007) The APOBEC-2 crystal structure and functional implications for the deaminase AID. Nature 445, 447–451

46. Chelico, L., Prochnow, C., Erie, D. A., Chen, X. S., and Goodman, M. F. (2010) Structural model for deoxycytidine deamination mechanisms of the HIV-1 inactivation enzyme APOBEC3G. J. Biol. Chem. 285, 16195–16205

47. Chelico, L., Sacho, E. J., Erie, D. A., and Goodman, M. F. (2008) A model for oligomeric regulation of APOBEC3G cytosine deaminase-dependent restriction of HIV. J. Biol. Chem. 283, 13780–13791

48. Zhang, H., Zhou, Y., Alcock, C., Kiefer, T., Monie, D., Siliciano, J., Li, Q., Pham, P., Cofrancesco, J., Persaud, D., and Siliciano, R. F. (2004) Novel single-cell-level phenotypic assay for residual drug susceptibility and reduced replication capacity of drug-resistant human immunodeficiency virus type 1. J. Virol. 78, 1718–1729

49. Langlois, M. A., Beale, R. C., Conticello, S. G., and Neuberger, M. S. (2005) Mutational comparison of the single-domain APOBEC3G and double-domain APOBEC3F/G anti-retroviral cytidine deaminases provides insight into their DNA target site specificities. Nucleic Acids Res. 33, 1913–1923

50. Naldini, L., Blomer, U., Gallay, P., Ory, D., Mulligan, R., Gage, F. H., Verma, I. M., and Trono, D. (1996) In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 272, 263–267

51. Creighton, S., Bloom, L. B., and Goodman, M. F. (1995) Gel fidelity assay measuring nucleotide misinsertion, exonucleolytic proofreading, and lesion bypass efficiencies. Methods Enzymol. 262, 232–256

52. Lieberman, B. A., and Nordeen, S. K. (1997) DNA intersegment transfer, how steroid receptors search for a target site. J. Biol. Chem. 272, 1061–1068

53. Luo, K., Liu, B., Xiao, Z., Yu, Y., Yu, X., Gorelick, R., and Yu, X. F. (2004) Amino-terminal region of the human immunodeficiency virus type 1 nucleocapsid is required for human APOBEC3G packaging. J. Virol. 78, 11841–11852

54. Schäfer, A., Bogerd, H. P., and Cullen, B. R. (2004) Specific packaging of APOBEC3G into HIV-1 virions is mediated by the nucleocapsid domain of the gag polyprotein precursor. Virology 328, 163–168

55. Savytskaia, E. S., Xu, H., Mbisa, J. L., Barr, R., Gorelick, R. I., Ono, A., Freed, E. O., Hu, W. S., and Pathak, V. K. (2004) Human apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G) is incorporated into HIV-1 virions through interactions with viral and nonviral RNAs. J. Biol. Chem. 279, 35822–35828

56. Zennou, V., Perez-Caballero, D., Göttingler, H., and Bieniasz, P. D. (2004) APOBEC3G incorporation into human immunodeficiency virus type 1 particles. J. Virol. 78, 12058–12061

57. Alce, T. M., and Popik, W. (2004) APOBEC3G is incorporated into virus-
like particles by a direct interaction with HIV-1 Gag nucleocapsid protein. J. Biol. Chem. 279, 34083–34086

58. Bach, D., Peddi, S., Mangeat, B., Lakkaraju, A., Strub, K., and Trono, D. (2008) Characterization of APOBEC3G binding to 7SL RNA. Retrovirology 5, 54

59. Wang, T., Tian, C., Zhang, W., Luo, K., Sarkis, P. T., Yu, L., Liu, B., Yu, Y., and Yu, X. F. (2007) 7SL RNA mediates virion packaging of the antiviral cytidine deaminase APOBEC3G. J. Virol. 81, 13112–13124

60. Strebel, K., and Khan, M. A. (2008) APOBEC3G encapsidation into HIV-1 virions: which RNA is it? Retrovirology 5, 55

61. Ooms, M., Majdak, S., Seibert, C. W., Harari, A., and Simon, V. (2010) The APOBEC3C crystal structure and the interaction interfaces for HIV-1 Vif binding. Nat. Struct. Mol. Biol. 17, 1005–1010

62. Li, M. M., Wu, L. I., and Emerman, M. (2010) The range of human APOBEC3H sensitivities to lentiviral Vif proteins. J. Virol. 84, 88–95

63. Tan, L., Sarkis, P. T., Wang, T., Tian, C., and Yu, X. F. (2009) Sole copy of Z2-type human cytidine deaminase APOBEC3H has inhibitory activity against retrotransposons and HIV-1. FASEB J. 23, 279–287

64. Li, M. M., Wu, L. I., and Emerman, M. (2010) The range of human APOBEC3H sensitivity to lentiviral Vif proteins. J. Virol. 84, 7961–7969

65. Ooms, M., Majdak, S., Seibert, C. W., Harari, A., and Simon, V. (2010) The localization of APOBEC3H variants in HIV-1 virions determines their antiviral activity. J. Virol. 84, 7961–7969

66. Apolonio, L., Schulz, R., Curk, T., Rocha, P., Swanson, C. M., Schaller, T., Ule, J., and Malim, M. H. (2015) Promiscuous RNA binding ensures effective encapsidation of APOBEC3 proteins by HIV-1. PLoS Pathog. 11, e1004609

67. Feng, Y., Love, R. P., and Chelico, L. (2013) HIV-1 viral infectivity factor (Vif) alters permissive single-stranded DNA scanning of the retroviral restriction factor APOBEC3G. J. Biol. Chem. 288, 6083–6094

68. Kitamura, S., Ode, H., Nakashima, M., Imahashi, M., Naganawa, Y., Kurosawa, T., Yokomaku, Y., Yamane, T., Watanabe, N., Suzuki, A., Sugira, W., and Iwatani, Y. (2012) The APOBEC3G crystal structure and the interface for HIV-1 Vif binding. Nat. Struct. Mol. Biol. 19, 1005–1010

69. Mu, Y., Prochnow, C., Pham, P., Chen, X. S., and Goodman, M. F. (2012) A structural basis for the biochemical behavior of activation-induced deoxy-cytidine deaminase class-switch recombination-defective hyper-IgM-2 mutants. J. Biol. Chem. 287, 28007–28016

70. Kohli, R. M., Abrams, S. R., Gajula, K. S., Maul, R. W., Gearhart, P. J., and Stivers, J. T. (2009) A portable hot spot recognition loop transfers sequence preferences from APOBEC family members to activation-induced cytidine deaminase. J. Biol. Chem. 284, 22898–22904

71. Carpenter, M. A., Rajagurubandara, E., Wijesinghe, P., and Bhagwat, A. S. (2010) Determinants of sequence-specificity within human AID and APOBEC3G. DNA Repair 9, 579–587

72. Rathore, A., Carpenter, M. A., Demir, Ö., Ikeda, T., Li, M., Shaban, N. M., Law, E. K., Anokhin, D., Brown, L. W., Amaro, R. E., and Harris, R. S. (2013) The local dinucleotide preference of APOBEC3G can be altered from 5'-CC to 5'-TC by a single amino acid substitution. J. Mol. Biol. 425, 4442–4454

73. Binka, M., Ooms, M., Steward, M., and Simon, V. (2012) The activity spectrum of Vif from multiple HIV-1 subtypes against APOBEC3G, APOBEC3F, and APOBEC3H. J. Virol. 86, 49–59

74. Ooms, M., Letko, M., Binka, M., and Simon, V. (2013) The resistance of human APOBEC3H to HIV-1 NL4–3 molecular clone is determined by a single amino acid in Vif. PLoS One 8, e57744

75. Senaviarthane, G., Jaszczyzcz, M., Auerbach, P. A., Upton, T. G., Chelico, L., Goodman, M. F., and Rueda, D. (2012) Single-stranded DNA scanning and deamination by APOBEC3G cytidine deaminase at single molecule resolution. J. Biol. Chem. 287, 15826–15835

76. Shlyakhtenko, L. S., Lushnikov, A. Y., Miyagi, A., Li, M., Harris, R. S., and Lyubchenko, Y. L. (2013) Atomic force microscopy studies of APOBEC3G oligomerization and dynamics. J. Struct. Biol. 184, 217–225

77. Krzyziak, T. C., Jung, J., Thompson, J., Baker, D., and Gronenborn, A. M. (2012) APOBEC2 is a monomer in solution: implications for APOBEC3G models. Biochemistry 51, 2008–2017

78. Holden, L. G., Prochnow, C., Chang, Y. P., Branstetter, R., Chelico, L., Sen, U., Stevens, R. C., Goodman, M. F., and Chen, X. S. (2008) Crystal structure of the anti-viral APOBEC3G catalytic domain and functional implications. Nature 456, 121–124

79. Shandilya, S. M., Nalam, M. N., Nalivaikia, E. A., Gross, P. J., Valesano, J. C., Shindo, K., Li, M., Munson, M., Royer, W. E., Harjes, E., Kono, T., Matsu, H., Harris, R. S., Somasundaran, M., and Schiffer, C. A. (2010) Crystal structure of the APOBEC3G catalytic domain reveals potential oligomerization interfaces. Structure 18, 28–38

80. Bohn, M. F., Shandilya, S. M., Albin, J. S., Kouno, T., Anderson, B. D., McDougle, R. M., Carpenter, M. A., Rathore, A., Evans, L., Davis, A. N., Zhang, J., Lu, Y., Somasundaran, M., Matsu, H., Harris, R. S., and Schiffer, C. A. (2013) Crystal structure of the DNA cytosine deaminase APOBEC3F: the catalytically active and HIV-1 Vif-binding domain. Structure 21, 1042–1050

81. Siu, K. K., Sultana, A., Azimi, F. C., and Lee, J. E. (2013) Structural determinants of HIV-1 Vif susceptibility and DNA binding in APOBEC3F. Nat. Commun. 4, 2593

82. Bohn, M. F., Shandilya, S. M., Silvas, T. V., Nalivaikia, E. A., Kouno, T., Kelch, B. A., Ryder, S. P., Kurt-Yilmaz, N., Somasundaran, M., and Schiffer, C. A. (2013) The ssDNA mutator APOBEC3A is regulated by cooperative dimerization. Structure 21, 903–911

83. King, J. J., Manuel, C. A., Barrett, C. V., Raber, S., Lucas, H., Sutter, P., and Larjiani, M. (2015) Catalytic pocket inaccessibility of activation-induced cytidine deaminase is a safeguard against excessive mutagenic activity. Structure 23, 615–627

84. Huthoff, H., Autore, F., Gallois-Montbrun, S., Fraternali, F., and Malim, M. H. (2009) RNA-dependent oligomerization of APOBEC3G is required for restriction of HIV-1. PLoS Pathog. 5, e1000330

85. Bogerd, H. P., Doehle, B. P., Wiegand, H. L., and Cullen, B. R. (2004) A single amino acid difference in the host APOBEC3G protein controls the primate species specificity of HIV type 1 virion infectivity factor. Proc. Natl. Acad. Sci. U.S.A. 101, 3770–3774

86. Mangeat, B., Turelli, P., Liao, S., and Trono, D. (2004) A single amino acid determinant governs the species-specific sensitivity of APOBEC3G to Vif action. J. Biol. Chem. 279, 14481–14483

87. Shandilya, S. M., Bohn, M. F., and Schiffer, C. A. (2014) A computational analysis of the structural determinants of APOBEC3's catalytic activity and vulnerability to HIV-1 Vif. Virology 471, 105–116

88. Aydin, H., Taylor, M. W., and Lee, J. E. (2014) Structure-guided analysis of the human APOBEC3-H-Vif restrictome. Structure 22, 668–684