Mutational comparison of the single-domained APOBEC3C and double-domained APOBEC3F/G anti-retroviral cytidine deaminases provides insight into their DNA target site specificities

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ABSTRACT

Human APOBEC3F and APOBEC3G are double-domained deaminases that can catalyze dC→dU deamination in HIV-1 and MLV retroviral DNA replication intermediates, targeting T–C or C–C dinucleotides, respectively. HIV-1 antagonizes their action through its vif gene product, which has been shown (at least in the case of APOBEC3G) to interact with the N-terminal domain of the deaminase, triggering its degradation. Here, we compare APOBEC3F and APOBEC3G to APOBEC3C, a single-domained deaminase that can also act on both HIV-1 and MLV. We find that whereas APOBEC3C contains all the information necessary for both Vif-binding and cytidine deaminase activity in a single domain, it is the C-terminal domain of APOBEC3F and APOBEC3G that confer their target site specificity for cytidine deamination. We have exploited the fact that APOBEC3C, whilst highly homologous to the C-terminal domain of APOBEC3F, exhibits a distinct target site specificity (preferring Y–C dinucleotides) in order to identify residues in APOBEC3F that might affect its target site specificity. We find that this specificity can be altered by single amino acid substitutions at several distinct positions, suggesting that the strong dependence of APOBEC3-mediated deoxycytidine deamination on the 5′-flanking nucleotide is sensitive to relatively subtle changes in the APOBEC3 structure. The approach has allowed the isolation of APOBEC3 DNA mutators that exhibit novel target site preferences.

INTRODUCTION

APOBEC3G (which has also been named CEM15) was discovered as an element capable of restricting infection of the non-permissive T-cell line CEM by Vif-deficient HIV-1 particles (1). In wild-type HIV-1, the virus-encoded Vif protein counteracts the anti-viral effect of APOBEC3G by inducing its poly-ubiquitination and subsequent proteasome-mediated degradation (2–6). An aspartic acid residue in the first domain of human APOBEC3G (position 128) is critical for the interaction with and sensitivity to Vif (7–10). In the absence of a Vif-interaction, APOBEC3G is encapsidated in HIV-1 [and can similarly be encapsidated by Moloney leukemia virus (MLV)] through binding to the retroviral gag polypeptide and, more specifically, to the nucleocapsid-forming region in the case of HIV-1 (11–15). The APOBEC3G protein that has been incorporated into the virion then acts in the target cell to deaminate deoxycytidines (dC) in the minus-strand retroviral cDNA replication intermediate (16–20). This deamination is not random: dC residues targeted by APOBEC3G are nearly always preceded by a 5′-dC residue (16,17,21,22).

Whilst evidence of retroviral restriction by APOBEC3G largely rests on in vitro co-transfection assays carried out with artificial retroviral constructs, support for the idea that host-mediated deamination of retroviral replication intermediates can actually occur during clinical HIV-1 infection is indicated by the observation that tonic G→A hypermutation (that can be ascribed to dC→dU deamination of minus-strand cDNA) is frequently observed in natural isolates of HIV-1 (23–27). However, the pattern of G→A substitutions exhibited by naturally occurring hypermutated HIV-1 sequences indicates that APOBEC3G is not the only cytidine deaminase that attacks HIV-1. Hypermutation is observed at both GG and GA dinucleotides in the retroviral genome consistent with...
deamination of both TC and CC dinucleotides in minus-strand cDNA. Indeed, recent work has revealed that other members of the human APOBEC3 family apart from APOBEC3G can act on HIV-1 (28–33). The human APOBEC3 family of cytidine deaminases comprises seven genes (A to D and F to H) that are encoded in a cluster located on chromosome 22 (34–37). In all cases, they contain either one or two copies of a core motif that is typical to all cytidine deaminases: \(\text{HXE-X_3}_{2-30}\text{-PXXXC}\) (34,38–40). Previous studies on \(E.\ coli\) cytidine deaminase (ECDDA) propose that the histidine and two cysteines in this motif enable Zn\(^{2+}\) coordination, whilst the glutamate serves as a proton donor in the deamination process (41–43). Whereas APOBEC3A, C and H have but a single zinc binding domain, APOBEC3B, -D, -F and -G are double-domain proteins, containing two copies of the zinc-coordination motif. Although the double-domain APOBEC3G is the most studied of these deaminases, it has not yet been fully resolved as to whether one of its two deaminase domains plays a dominant role in retroviral restriction/deamination (17,20,44).

Recent evidence has now revealed that, in addition to APOBEC3G, the double-domain deaminases APOBEC3B and APOBEC3F can also be incorporated into HIV-1 virions and deaminate retroviral minus-strand cDNA (28–31,33). Indeed, their mutation spectra could account for much, if not all, of the hypermutation seen at TC dinucleotides (28–31,33).

With regard to single domain APOBEC3’s, we have previously shown that human APOBEC3C (which is highly homologous to the second domain of human APOBEC3F) is capable of deaminating dC in DNA as judged by a bacterial mutagenesis assay (21). Whilst APOBEC3C did not exhibit any strong restricting activity against HIV-1 or MLV in earlier comparisons of the anti-retroviral activity of APOBEC3 family members (28–31,33), recent results from Yu \textit{et al.} (29) reveal that APOBEC3C can act as a potent inhibitor of SIV infection. In fact, we find that whilst APOBEC3C is certainly less potent than APOBEC3F and APOBEC3G, it can clearly act to deaminate MLV (as well as HIV-1) retroviral replication intermediates, with a characteristic target site preference. This has allowed us to use an MLV-based assay to perform a mutagenesis and domain-swap comparison of APOBEC3C and APOBEC3F that give insight into amino acid residues that affect their target site specificity.

MATERIALS AND METHODS

Cells and vectors

The CEM-SS cell line was obtained from the National Institute for Biological Standards and Control, Herts, UK and maintained in RPMI supplemented with 10% fetal calf serum (FCS), penicillin and streptomycin. 293T cells were cultured in DMEM supplemented with 10% FCS, penicillin and streptomycin. Whilst 293T cells express endogenous APOBEC3C and APOBEC3F (but not APOBEC3G) as judged by RT–PCR, northern blot analysis reveals that the abundance of these transcripts is very low and is augmented several orders of magnitude following transfection. MLV and HIV-1-like viruses were generated using a three-plasmid expression system employing a HIV-1 or MLV shuttle vector, a Gag-Pol expresser and a vector encoding the envelope glycoprotein VSV-G. The self-inactivating HIV-1 packageable vector CSGW encodes eGFP expressed from a SFFV 5’LTR promoter (45,46). HIV-1 Gag-Pol expression plasmids with (p8.2) or without (p8.9) vif were previously described (47).

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3C: (F-KpnI) 5'-NNN \\

3F1: (F-XhoI) 5'-NNN \\

3F2: (F-KpnI) 5'-NNN \\

3G1: (F-XhoI) 5'-NNN \\

3G2: (F-KpnI) 5'-NNN \\

3H1: (F-KpnI) 5'-NNN \\

3H2: (F-KpnI) 5'-NNN \\

RT–PCR

RNA was extracted from CEM or CEM-SS cells using TRIZOL reagent (Gibco) according to the manufacturer’s specifications. Ten micrograms of RNA were reverse-transcribed using random hexamer (NEB) and Superscript II reverse transcriptase (Invitrogen). APOBEC3F was amplified using the following primers: (F): 5'-CCTACCCAGAC-CTATGTGGAAAC-3', (R): 5'-CAGTAGTGCAGTAGAACAGGAAAGC-3'.
(R): 5'-CTGACATCTTCTGTGATCATCAG-3'. These primers are specific for APOBEC3F and APOBEC3G and did not cross-react. APOBEC3C was amplified with the following primers: (F): 5'-GGAGCCACCATGAATCCACAGATCAGAAA-3', (R): 5'-TCCTGTTACATGGATACCTG-3'.

Generation of virus-like particles

293T cells were co-transfected with Fugene 6 (Roche) at 70% confluence. FLAG- or eGFP-APOBEC encoding plasmids were transfected at a 0.2:1 ratio with respect to M5P and at a 1:1 ratio with respect of M5P unless otherwise stated. For HIV-1 infectivity assays, cells were co-transfected with CSGW, p8.2 or p8.9 and pMDG at a 1:0.75:0.75 ratio. For MLV production, the cells were transfected with M5P, pOGP and pMDG at a 1:0.70:0.30 ratio. All transfections within the same experiment were made-up to the same amount of total DNA using pcDNA3.1 (Invitrogen). 293T cells were washed the day following transfection with PBS and grown in culture media for 48 h. Virus-containing supernatants were collected 72 h post-transfection and filtered through a 0.45 μM filter.

Encapsulation

Viruses were produced as described in the previous section by co-transfection of FLAG-APOBEC and virus expression vectors. Supernatants were collected 72 h post-transfection and 293T packaging cells were harvested and assayed for FLAG-APOBEC expression by immunoblot. Virus-containing supernatants were pelleted through a 20% sucrose-PBS solution by ultracentrifugation at 175 000 g in a Beckman TLA-100.4 rotor for 1 h as described elsewhere (9). Virus pellets were solubilized in RIPA buffer containing 0.5% NP-40. Virion and cell lysates were analysed by western blot using a HRP-conjugated anti-FLAG antibody (Sigma).

Viral sequence analysis

293T target cells were collected 48 h post-infection and DNA was extracted using Puregene reagent (Gentra Systems) according to the manufacturer’s specifications. DNA preparations were then treated with the restriction endonuclease DpnI to eliminate any trace of plasmid carry-over from the transfection. DNA from MLV or HIV-1 infected cells were amplified using the high fidelity polymerase Pfu turbo (Stratagene) using the following primers: MLV: (F): 5'-ATCCCGGCGGTCACGAAC-3', (R): 5'-ATGGTGAGCAAGGGCGAGGAG-3'; HIV-1: (F): 5'-ATGGTGAGCAAGGGCGAGGAG-3'; (R): 5'-ATCCCGGCGGCGTACCAAC-3'. PCR products were then gel purified and cloned using the TOPO-Blunt cloning kit (Invitrogen) and sequenced using M13 reverse primers.

Southern blot

DNA was prepared from target 293T cells as described above. Total DNA was then digested with BamHI and NotI restriction endonucleases and resolved on a 1.2% agarose gel. DNA was then transferred by capillarity to a Hybond N+ nylon membrane and hybridized with a 32P-labeled eGFP cDNA probe for 16 h at 65°C. Membranes were washed three times for 30 min in 0.1% SDS, 0.2x SSC and analyzed by phosphorImager.

Immunoprecipitations and western blots

For protein analysis, cells were lysed in 10 mM Tris–HCl pH 8, 150 mM NaCl, 10% glycerol, 1% Nonidet P-40 supplemented with protease inhibitors (Roche). EGF fusion proteins were immunoprecipitated using rabbit anti-GFP (5 mg/ml) (Abcam) together with protein A-Sepharose beads (Amersham). EGF fusions were visualized by western blot using a HRP-conjugated anti-GFP antibody at a 1:8000 dilution (Abcam), whereas Vif was detected using a 1:1000 dilution of a monoclonal anti-Vif antibody (NIBSC, South Mimms, UK).

RESULTS

Anti-retroviral activity of APOBEC3C

Initial experiments revealed that APOBEC3C (like APOBEC3F and APOBEC3G, but distinct from the related cytidine deaminase AID) could be incorporated into MLV virus-like particles (Figure 1A). This incorporation can indeed have functional implications since APOBEC3C is able to inhibit MLV infection and trigger G→A mutation of viral replication intermediates (Figure 1B and C). The spectrum of the mutations reveals that the target specificity is distinct from that of both APOBEC3G (which essentially acts only on CC dinucleotides) and APOBEC3F (which essentially acts only on TC dinucleotides): APOBEC3C targets dCs that are preceded by either dC or dT. In contrast, expression of the AID deaminase in these assays gave little anti-viral activity above that of the vector-only control and did not yield any detectable mutation of MLV replication intermediates (Figure 1B, bottom).

The precise mechanism by which the APOBEC3 proteins restrict retroviral infectivity under physiological circumstances is uncertain. Various mechanisms have been suggested. These include the possibilities that diminished infectivity is simply a consequence of an excessive mutation load as well as the suggestion that the incorporation of uracil into the retroviral minus-strand cDNA might trigger its destruction (16,48,49). We were therefore interested in ascertaining whether APOBEC3C affected the persistence of viral DNA following infection. Southern blot analysis revealed that all three APOBEC3 members analyzed effected a substantial decrease in the extent of retroviral DNA integration in the infected cells using an MLV-based assay system, although the three deaminases yielded substantially different loadings of G→A mutation in the retroviral replication intermediates under the same assay conditions (Figure 1D). Although we cannot discriminate whether the viral DNA detected in the Southern blots derives from sequences that have or have not integrated into a host chromosome, the results nevertheless reveal that co-transfection with APOBEC3 plasmids leads to a diminished abundance of retroviral DNA in the target cell, presumably reflecting degradation.

It is clearly interesting to know whether APOBEC3C can also function as a restriction barrier to HIV-1 infection. We find that the APOBEC3C does exert an anti-viral effect in an HIV-1-based assay system (similarly giving rise to G→A mutation of viral replication intermediates) although APOBEC3C-encoding plasmid is again somewhat less potent than the APOBEC3F/G plasmids. This restriction of infection
is at least in part relieved by the HIV-1 Vif gene product (Figure 2A). Furthermore, cytofluorometry experiments performed using cells transected with plasmids expressing eGFP-tagged APOBEC3C with or without Vif-expressing plasmids indicates that Vif does indeed mediate the destabilization of APOBEC3C (Figure 2B). This destabilization of APOBEC3C by Vif is somewhat surprising in view of the fact that APOBEC3C bears high (81%) homology to the C-terminal domain of APOBEC3F whereas mutational/homology considerations (7–10,34) make it likely that it is the N-terminal domain of APOBEC3F (to which APOBEC3C shows only 53% homology) that interacts with Vif. In order to ascertain whether the Vif-mediated destabilization does indeed correlate with a Vif-APOBEC3C interaction, we performed immunoprecipitation experiments. An interaction can indeed be seen between the two proteins (Figure 2C).

If restriction of HIV-1 infection by endogenous (as opposed to transfected and over-expressed) APOBEC3C also applies in vivo, then one might expect APOBEC3C not to be expressed in cell-lines that are permissive for infection by Δvif HIV-1 particles. Indeed, the original discovery that an APOBEC3 family member acted as retroviral restriction elements was based on the observation that Δvif HIV-1 can be propagated in CEM-SS cells (a permissive T-cell line) but not in the non-permissive CEM progenitor (1). RT–PCR analysis reveals that APOBEC3C, APOBEC3F and APOBEC3G are all expressed in CEM cells but that the expression of all three of them is indeed lost in the CEM-SS derivative (Figure 2D).

The second domain of APOBEC3F and of APOBEC3G encode the elements responsible for deamination specificity

Since it is the second domain of APOBEC3F that is homologous to the single-domain APOBEC3C, we wondered if the catalytic specificity of APOBEC3F resided in its second domain. One way to approach this issue (as it has been performed with APOBEC3G) is to analyse whether mutations likely to affect zinc coordination by either of the two domains affects its catalytic activity. However, a problem with such an approach is that retention of activity could mean that the catalytic domain in question had not been inactivated; loss of activity could be through general protein destabilization or degradation. Therefore, in view of the fact that APOBEC3C, APOBEC3F and APOBEC3G all exhibit different target specificities, we asked whether replacing the second domain of APOBEC3F by that of APOBEC3G or by APOBEC3C could alter the target specificity of APOBEC3F. All chimeras were readily expressed in 293T cells (Figure 3A) and gave similar inhibition of viral infection as APOBEC3C (Figure 3B and Table S2). The results on DNA deamination were straightforward. The target specificities of the resultant double-domain APOBEC3 chimeras all accorded with that of the C-terminal domain (Figure 3C).

An APOBEC3F second domain/APOBEC3C chimera yields a novel target specificity

In order to further dissect the parts of the second domain of APOBEC3F that mediate target specificity, we generated variants of APOBEC3F (designated F1-Ca/F2b and F1-F2a/Cb, respectively) in which the N- or C-terminal halves of the APOBEC3F second domain were substituted by corresponding portions from APOBEC3C. To our surprise, whilst both chimeras were active in restricting MLV infection, they yielded a mutation spectrum that was quite distinct from both the APOBEC3F and APOBEC3C parents. They both exhibited a strong bias for a C at position −1 (similar, although not quite as marked in extent, to the preference displayed by APOBEC3G) (Figure 3D and Table S2).

Effects of single amino acid substitutions in the second domain of APOBEC3F on target specificity

In order to gain more insight into the molecular basis of the target site specificity of the APOBEC3 deaminases, we tested the effects on specificity of single amino acid substitution in the C-terminal domain of APOBEC3F (Figure 4A and Table S3). The nature of the mutations made was guided by a comparison of the sequence of APOBEC3C with that of APOBEC3F. Thus, mutations were made in residues that differed between APOBEC3F and APOBEC3C, so as to identify those that could be responsible for a shift from the typical T deamination spectrum of APOBEC3F to the Y_C spectrum of APOBEC3C. Of the 12 single amino-acid substitutions tested, only APOBEC3F mutant 7 substantially altered the deaminated spectrum at −1, whilst mutants 6 and 9 both gave some modification to the spectrum at position −2 (Figure 4B and Table S3).

DISCUSSION

The results of this study clearly reveal that APOBEC3C can work on both MLV and HIV-1. An antiviral effect of

Figure 1. APOBEC3C has an anti-retroviral effect on MLV. (A) APOBEC3 proteins but not AID or U2AF are packaged in MLV virus-like particles. Virions were produced by co-transfection of 293T cells with M5p, Gag-Pol, VSV-G and Flag-tagged APOBEC expression vectors. Three days after transfection, viruses were isolated from the supernatant by ultracentrifugation and producer cells were collected. Viral pellet and producer cells were then lysed and submitted to western blot analysis using anti-FLAG HRP-conjugated monoclonal antibody. Sample loading was controlled using an anti-p30gag antibody. (B) Upper panel: inhibition of MLV infection by various AID or APOBEC3 proteins. 293T cells were co-transfected with MLV producer plasmids in combinations with either of the FLAG-tagged APOBEC3s. Of the 12 single amino-acid substitutions tested, only APOBEC3F mutant 7 substantially altered the deaminated spectrum at −1, whilst mutants 6 and 9 both gave some modification to the spectrum at position −2 (Figure 4B and Table S3).
Figure 2. APOBEC3C inhibits HIV infection and is sensitive to Vif. (A) Left panel: inhibition of HIV-1 and HIV-1 (ΔVif) by various AID or APOBEC3 proteins. 293T cells were co-transfected with HIV-1 or HIV-1 (ΔVif) producer plasmids in combinations with either of the FLAG-tagged APOBEC3 or of the AID expression plasmids. Histograms represent the mean of at least three independent experiments and error bars represent the corresponding standard error. Right panel: titration effect of various FLAG-tagged APOBEC3 proteins on HIV-1 (ΔVif) infectivity. Increasing APOBEC3/AID:CSGW ratios were used to co-transfect 293T cells. Each point represents the average of two independent experiments. *Significantly different by Student’s t-test (P = 0.007). (B) 293T cells were co-transfected with pcGFP-C3, eGFP-APOBEC3C or eGFP-APOBEC3G and plasmids expressing either Vif or ΔVif (Ctrl). Fluorescence was monitored for 5 days by FACS analysis. Each point represents the average and standard error of three independent measurements. (C) 293T cells were co-transfected with pVif alone (Ctrl) or pVif and pcGFP-C3, eGFP-APOBEC3C or eGFP-APOBEC3G, and cell lysates were prepared 48 h later. The bulk of the lysate was submitted to immunoprecipitation with an anti-eGFP monoclonal antibody. Immunoprecipitates and cell lysates were then submitted to SDS-PAGE and the western blots were probed with anti-Vif monoclonal antibodies. (D) Expression of APOBEC3C, APOBEC3F and APOBEC3G in CEM and CEM-SS cells. An RT-PCR was performed on RNA isolated from either CEM (upper panel) or CEM-SS (lower panel). Results reveal that the CEM-SS clone has lost the expression of APOBEC3C, -3F and -3G.
APOBEC3C on MLV and/or HIV-1 is in fact apparent in several (but not all) previous studies (28–30,33). Whilst differences in the assay systems and retroviral substrates used in the various studies might be a major contributing factor, we suspect that the activity of APOBEC3C against MLV and HIV-1 has not been previously highlighted because it is evidently less potent against these retroviruses than are APOBEC3F and APOBEC3G. Strikingly, in a recent study, Yu et al. (29) have noted that APOBEC3C is very potent against SIV.

Figure 3. The second domain of APOBEC3F and APOBEC3G enclose the elements governing deamination spectrum specificity. (A) Western blot analysis of the different chimeric APOBEC3 proteins. An HRP-conjugated anti-eGFP antibody was used to reveal the bands. (B) F1C and F1G2 chimeric APOBEC3 proteins show a strong antiviral effect on MLV virus-like particles. Histograms represent the mean of three independent experiments and error bars represent the standard error. (C and D) Schematic representation of the different chimeric APOBEC3F proteins and their respective deamination spectrum at positions −2 and −1 in relation to the deaminated dC is depicted in pie charts. ‘n’ represents the number of independent G→A mutations.
The in vitro results do not, of course, prove that APOBEC3C acts as a viral restriction element in vivo. This is a difficult issue to address by asking whether APOBEC3C leaves a footprint on the mutation spectrum of naturally occurring hypermutated HIV-1 sequences since the target specificity of APOBEC3C is likely to show some overlap with that of APOBEC3F. However, it is notable that co-transfection with Vif can overcome APOBEC3C-mediated restriction of HIV-1 (29) and, as we show here, Vif and APOBEC3C can partake in an interaction that correlates with APOBEC3C degradation. This is somewhat remarkable in light of the fact that, at least in the case of APOBEC3G, distinct domains seem to be largely responsible for Vif binding and for dominant catalytic activity [(6–10) and see below].

Despite inducing mutations at a frequency several-fold lower than APOBEC3G or APOBEC3F, APOBEC3C is still capable of inhibiting viral infection and blocking viral DNA persistence in the target cells. The precise mechanism by which APOBEC3 proteins inhibit retroviral infection is not known, but it is possible that the extent of inhibition of retroviral integration does not correlate simply with the extent of retroviral deamination. Interestingly, although G→A mutations in the HBV genome have been observed in certain experimental conditions and also in clinical samples (50,51), it has been proposed by Turelli et al. (52) that APOBEC3G-mediated inhibition of HBV infection could be independent of deamination. Somewhat analogous observations have been made by Newman et al. with regard to HIV-1 (53). These

Figure 4. The deamination spectrum of APOBEC3F is altered by single amino acid substitutions. (A) Sequence alignment of APOBEC3C and the second domain of APOBEC3F. Numbers indicate residues that were changed to generate the 12 mutant APOBEC3F proteins. (B) Histograms depicting the proportion of each nucleotide at positions –2 and –1 in relation to the deaminated dC.
results hint that there may be other (not deamination-based) mechanisms by which APOBEC3 proteins hinder viral infection.

It has been somewhat unclear as to whether catalytic activity is contained within either or both the domains of the double-domained deaminases (17,20,44). In studies of APOBEC3G, several single or double amino acid substitutions have been found to lead to diminished accumulation of G—A mutations in the retroviral target although, as noted by Shindo et al. (44), this diminished activity can often be correlated with diminished protein stability, in particular when working with truncated forms of these deaminases (6). Interestingly, however, none of the amino acid substitutions generated in APOBEC3G to date have been found to be sufficient to destroy all deaminating activity although mutations within the catalytic site of the second domain were found to lead to a complete loss of antiviral activity (17,20,44). Here, we have investigated the target site specificities of various chimeric APOBEC3s in order to identify which parts of the proteins are implicated in catalytic activity. The results reveal that the second domain of APOBEC3F and APOBEC3G play a critical role in determining the target specificity of these deaminases with regards to sensitivity to 5’-flanking nucleotides. It is difficult to imagine any interpretation other than that these domains are catalytically active, although we obviously cannot exclude the possibility that the N-terminal domains of these deaminases also exhibit some (but probably much lesser) activity. [During the course of this work, we also generated chimeras in which the N-terminal domain of APOBEC3G was fused with the C-terminal domain of APOBEC3F or with APOBEC3C, but these proteins turned out to be unstable (data not shown) and therefore gave no insight into the possible catalytic activity of the N-terminal domains.]

We also generated deaminases carrying a chimeric C-terminal domain composed of part APOBEC3C and part APOBEC3F-second domain in order to gain insight into the different target site preferences conferred by these two domains. To our surprise, the hybrids exhibited a target specificity closer to that of APOBEC3G than that of either parent. Furthermore, analysis of APOBEC3F point mutants reveal that sensitivity to 5’-flanking nucleotides can be modified by distinct single amino acid substitutions. Whilst a definitive interpretation of these mutations must await the elucidation of the three-dimensional structure of APOBEC3 deaminases, the results demonstrate that target site preference can be easily perturbed and strongly suggest that this preference must be sensitive to relatively subtle changes in deaminase structure.

It is striking that all four antiviral APOBEC3 deaminases (APOBEC3B, -3C, -3F and 3G) that have been characterized to date display different DNA target site specificities. The precise significance of retroviral deamination to the physiological functioning of the APOBEC3 proteins is still uncertain. It is therefore difficult to know whether the differing target site preferences of the various APOBEC3 proteins reflect differences that were selected during evolution because the local targeting of deamination is intimately associated with the functioning of these proteins in restricting viral infection (35,54). An alternative possibility, given the ease with which the target site preference is perturbed, is that the differing mutational specificities of these proteins are incidental by-products of changes, possibly located outside the immediate vicinity of the active site, that have been selected for other reasons (e.g. a modulation of protein–protein interactions so as to avoid viral counter-measures).

It is interesting in this context to compare the target site preference of the APOBEC3 proteins to that of AID (activation-induced deaminase) which functions in the adaptive immune system to trigger antibody gene diversification (55,56). AID has a quite distinct target site preference from the APOBEC3 proteins, preferring a purine upstream of the targeted dC (57–59). The deamination preference of AID will presumably have co-evolved with the sequences of its physiological targets (the variable and switch regions of the immunoglobulin loci) so as to allow both optimal targeting of somatic mutations during antibody affinity maturation and efficient class switch recombination (60,61). The structural basis for major difference in target specificity of AID and APOBEC3 proteins is currently unknown. Strikingly, in a recent mutagenesis study of AID, Bransteitter et al. (62) have similarly concluded that a single amino acid substitution outside the catalytic site (in fact located in the N-terminal region of this protein) can affect mutational specificity.

Both this study and that of Bransteitter et al. (62) reveal that it is relatively easy to generate altered AID/APOBEC family deaminases that exhibit novel mutational specificities. AID-mediated DNA deamination not only underpins the physiological mechanism of antibody affinity maturation, it also underpins several in vitro strategies that exploit an iterative alternation of mutation and selection to derive proteins with desired characteristics (63–65). The functional diversity in these in vitro systems is significantly restricted by the limited mutational specificity of the DNA deaminase: generating a range of deaminases with differing (and possibly more relaxed) target specificities could therefore prove of some utility.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at NAR Online.

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