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# Diverse functions for DNA and RNA editing in the immune system

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Polynucleotide DNA and RNA editing enzymes alter nucleic acid sequences and can thereby modify encoded informational content. Two major families of polynucleotide editing enzymes, the AID/APOBEC cytidine deaminases (which catalyze the deamination of cytidine to uridine) and the adenosine deaminases acting on RNA (ADARs, which catalyze the deamination of adenosine to inosine), function in a variety of host defense mechanisms. These enzymes act in innate and adaptive immune pathways, with both host and pathogen targets. DNA editing by the cytidine deaminase AID mediates immunoglobulin somatic hypermutation and class switch recombination, providing the antibody response with the flexibility and diversity to defend against an almost limitless array of varied and rapidly adapting pathogenic challenges. Other cytidine deaminases (APOBEC3) restrict retroviral infection by editing viral retrogenomes. Adenosine deaminases (ADARs) shape innate immune responses by modifying host transcripts that encode immune effectors and their regulators. Here we review current knowledge of polynucleotide DNA and RNA editors with a focus on these and other functions they serve in the immune system.

Biological information is coded in the base sequence of DNA and RNA. It follows that the fidelity of this information is meticulously preserved during its replication, transcription and maintenance, particularly in higher organisms; alterations at the level of genome or transcriptome can have dramatic downstream functional implications. Unintended sequence changes often lead to deleterious consequences. However, despite the pressure to guard against such effects, many biological systems have developed targeted mechanisms to alter DNA or RNA sequences and their corresponding information content. Two primary categories of enzymes that edit polynucleotide sequences are the cytidine deaminases (which catalyze deamination of cytidine to uridine in RNA or DNA) and adenosine deaminases (which catalyze deamination of adenosine to inosine in RNA). Although such sequence modifications have diverse effects throughout biology, many provide important functions in host defense.

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The immune system faces a virtually limitless array of pathogens with correspondingly diverse structures and functions. In order to combat potential threats, the host must distinguish "self" from "non-self" targets and direct effector mechanisms accordingly. Innate immune defenses typically recognize common pathogen-associated molecular patterns (PAMPs) and/or molecular elements normally alien to the eukaryotic cell (e.g., viral replication intermediates). The germline-encoded receptors of the innate immune system mediate many key aspects of host defense, but are alone insufficient to defend against all potential pathogens; individually encoded receptors specific to each distinct threat would require a genome of massive size and complexity. Furthermore, pathogens can rapidly adapt to and evade host defenses, necessitating a variable and adjustable response. The adaptive immune system addresses these problems through mechanisms that introduce considerable genetic diversity within the antigen receptor genes of immune cells. In conjunction with cellular selection processes, these processes provide a flexible antigen recognition system while allowing for a host genome of reasonable size.

Pathogens employ many strategies to subvert host functions, which demand similarly diverse host defenses. As instruments of informational diversity, polynucleotide DNA and RNA sequence editors are important players in innate and adaptive immunity. In particular, the AID/APOBEC family of cytidine deaminases and ADAR family of adenosine deaminases mediate various immune functions. Their targets include the genomes and transcriptomes of both host and pathogen. Host-directed mRNA editing shapes innate immunity by altering the content of transcripts coding for effector proteins (ADARs). Editing directed at retroviral sequences represents a potent innate defense that can mutate and thereby cripple viral genomes (APOBEC3). Meanwhile, DNA editing at host immunoglobulin (Ig) loci is critical in establishing the genetic diversity required for an effective humoral adaptive immune response (AID). Not surprisingly, some pathogens have evolved means to harness host editing mechanisms to enhance their own functions (ADARs). Here we review current knowledge of polynucleotide DNA and RNA editors with a focus on these and other functions they serve in the immune system.

## AID/APOBEC Cytidine Deaminases in the Immune System

**APOBEC1, an RNA-editing cytidine deaminase.** The first mammalian polynucleotide cytidine deaminase identified,[1,2]

Apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1 (APOBEC1) is an mRNA editing enzyme with a well-characterized function in lipid absorption. It mediates the tissue-specific and splicing-independent regulation of apolipoprotein B (apoB) isoforms. The full-length apoB-100 isoform is a principal component of hepatic low-density lipoprotein (LDL) particles, while the truncated apoB-48 isoform is predominant in the chylomicrons generated in the small intestine. Transcription and splicing of the apoB mRNA is identical in both tissues. However, in the small intestine, APOBEC1 catalyzes the site-specific deamination of C6666, effectively converting a CAA glutamine codon to a UAA stop codon. Upon translation, apoB-48 is produced and subsequently incorporated into chylomicrons with absorbed dietary lipid.

ApoB editing requires APOBEC1 complementation factor (ACF), an RNA binding protein and component of the APOBEC1 "editosome".[3] ACF recognizes and binds to an 11 nt "mooring sequence" downstream (3') of the edited C, and is thought to position the edited base appropriately in the APOBEC1 active site. ACF also functions to suppress nonsense-mediated decay of the edited apoB transcript.[4]

At present, apoB represents the only known physiological APOBEC1 mRNA editing target in healthy tissue. However, there is some evidence that APOBEC1 may also function in the immune system. As an RNA binding protein, APOBEC1 associates with and stabilizes several AU-rich mRNAs, thereby influencing their translation independently of editing activity.[5-7] One such mRNA binding substrate, COX-2,[6] is a key factor in prostaglandin-mediated inflammation. Furthermore, though APOBEC1 expression was long considered exclusive to the intestine, some evidence indicates the presence of APOBEC1 transcripts in mouse secondary lymphoid tissues[8] and several immune cell types (Rosenberg BR and Papavasiliou NF, unpublished data). Further study of potential APOBEC1 targets in intestine and/or leukocytes may reveal novel functions in the immune system.

**Activation-induced cytidine deaminase drives antibody diversification.** Though APOBEC1 was identified because of its function in lipid absorption, the discovery of Activation-induced cytidine deaminase (AID) demonstrated a fundamental role for polynucleotide cytidine deaminases in the immune system.

AID is a central mediator of the adaptive immune response, driving antibody diversification in response to antigenic challenge through two distinct processes: somatic hypermutation (SHM) and class switch recombination (CSR). In SHM, point mutations are introduced within the V(D)J regions of rearranged Ig loci, thereby altering the antigen binding properties of the encoded Ig. SHM occurs in germinal center B cells and those B cells that acquire improved antigen binding affinity are positively selected for survival. During this "affinity maturation," the Ig variable regions accumulate additional mutations and the activated B cells are thereby optimized for a highly specific and potent adaptive immune response. As these modifications are fixed in somatic B cell genomes, they are maintained in clonal expansion and subsequent memory responses. In CSR, a region specific recombination reaction replaces the primary constant (Cμ) region with a downstream constant region (Cγ, Cε or Cα). Constant regions code for the Fc portions of antibody molecules; recombination between these regions changes the isotype and endows the antibody protein with different effector properties. This genomic and functional flexibility allows for an antigen-appropriate response in many different infection contexts. AID is necessary for both of these processes.[9]

AID was identified in a subtractive hybridization screen comparing resting B cells and B cells stimulated to undergo CSR.[10] Initial sequence analysis of AID revealed that it contains a cytidine deaminase domain with considerable homology to the previously characterized RNA editor, APOBEC1. As such, it was initially hypothesized that AID also edited mRNA. To account for the involvement of AID in both SHM and CSR, it was thought that AID might target two distinct mRNAs, such as a transcript encoding a DNA mutator for SHM and a transcript encoding a region-specific recombinase for CSR. Alternatively, AID might edit a single mRNA encoding a protein that functions in both SHM and CSR. Either mechanism would require task-specific and/or targeting cofactors, similar to the APOBEC1/ACF model.[3,9] This proposal is referred to as the "RNA editing hypothesis."

In the alternative "DNA editing hypothesis," AID acts on and mutates DNA directly. This model fits with earlier theories predicting the existence of a mutating factor that directly targets the Ig locus.[11-13] Additional characterization demonstrated that ectopically expressed AID could mutate the genomes of several mammalian cell types,[14,15] yeast[16] and bacteria.[17] It is unlikely that AID would edit the same mRNA in both prokaryotic and eukaryotic cells in order to code for a novel DNA mutator.

A variety of DNA repair mechanisms were also shown to contribute to both SHM and CSR, further supporting the editing model in which AID acts directly on the genome. As a cytidine deaminase, AID activity was predicted to introduce U:G mismatches in DNA. Such mismatches are typically resolved by Uracil DNA glycosylase (UNG), the primary effector of uracil removal in base excision repair. Interestingly, UNG-deficient animals acquire Ig locus mutations at rates comparable to wild-type controls.[18] However, the spectra of mutations in the absence of UNG are dramatically different. While mutation patterns at A and T bases are similar, mutations at G and C residues are strongly biased towards G to A and C to T events in $UNG^{-/-}$ mice, thereby implicating genomic uridines and their repair in shaping the SHM profile. Aside from SHM, UNG (in combination with the mismatch repair enzyme Msh2) is also necessary for CSR.

Though beyond the scope of this review, numerous additional studies of DNA damage and repair mechanisms in the context of CSR and/or SHM further demonstrated that AID acts directly on genomic cytidines. In SHM, AID deaminates cytidines within rearranged Ig V(D)J segments (**Fig. 1A**). The recognition and error-prone resolution of these deoxyuridines by the cellular repair machinery leads to the transition and transversion point mutations of B cell affinity maturation. In CSR, AID deaminates cytidines within repetitive sequences between the variable and constant regions of Ig gene segments (**Fig. 1B**). This editing event (and corresponding DNA breaks) initiates the region-specific

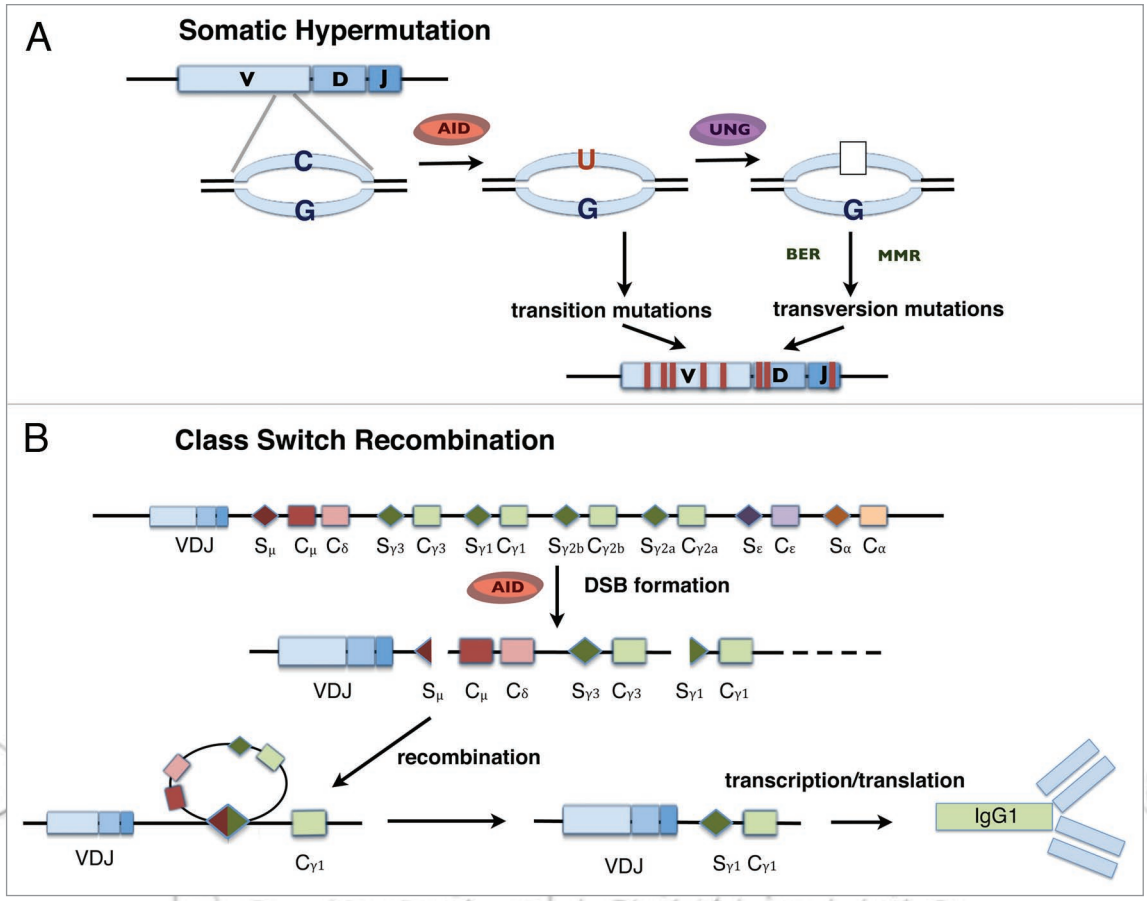




**Figure 1.** AID drives antibody diversity through two distinct mechanisms. (A) In SHM, AID deaminates cytidines within the variable region of Ig loci. Genomic deoxyuridine residues are then resolved by two pathways. Uridine is read as thymidine by replication machinery, leading to transition mutations. Alternatively, UNG excises the edited base, which is followed by abasic site repair via base-excision repair (BER) and mismatch-repair (MMR) enzymes, leading to transversion mutations. (B) In CSR, AID deaminates cytidines within Ig switch (S) regions, leading to double strand breaks. Recombination replaces the primary constant switch region (Cμ) with one of several downstream constant regions (Cγ, Cε or Cα), altering the effector properties of the encoded antibody. In this representation, the primary Cμ region is replaced with a $C\gamma_1$ region, thereby causing a switch from the IgM to the $IgG\gamma_1$ isotype.

recombination reaction that results in Ig constant region isotype switch. Despite homology to APOBEC1, the majority of available data supports this model of AID as a DNA editor. In this capacity, it plays a central role in adaptive immunity by generating the antibody diversity necessary for host defense against an innumerable variety of antigenic challenges.

**AID and cellular transformation.** Despite benefits in host defense, genomic DNA editing carries significant risks. In the context of CSR, it is thought that AID deamination (together with UNG activity) generates coupled DNA breaks at the Ig locus. Most of these are appropriately repaired, leading to deletional recombination. However, occasionally the breaks may be erroneously resolved, giving rise to chromosomal translocations. The partner loci for such rearrangements can be either genomic sites that can serve as infrequent off-site targets for AID (including many proto-oncogenes[19,20]), or hypersensitive sites susceptible due to structure (e.g., cruciform DNA at the bcl-2 locus[21]) or function (e.g., enhancer elements). Incorrect break resolution can be caused by deficiencies of DNA repair factors[22] or lack of Ig locus CSR targeting elements.[23]

AID-mediated chromosomal translocations have been implicated in the oncogenesis of B cell lymphomas. However, AID may also contribute to tumorigenesis in non-lymphoid tissues. Studies from several groups have shown that AID expression is not restricted to germinal center B cells. AID is expressed in bone marrow pre/pro B cells after infection with a transforming virus,[24] in breast and ovarian tissue upon estrogen stimulation,[25] in prostate cells upon androgen receptor signaling,[25] and in a variety of human malignancies.[26] Though it remains unclear how expression is induced in each case, it seems that AID may affect tumor pathogenesis and development. Recent data indicate that AID can participate in the epigenetic reprogramming of cells to undifferentiated states.[27] Other work has shown that AID activity can contribute to chemotherapeutic resistance and tumor relapse.[28] Oncogenic events represent an infrequent but deleterious outcome of dysregulated and/or mistargeted DNA editing. In the case of AID, the benefit of antibody diversification to host defense generally outweighs these consequences of off-target mutation.

**APOBEC3G restricts HIV infection.** Detailed functional characterization of APOBEC1 and AID provided a framework

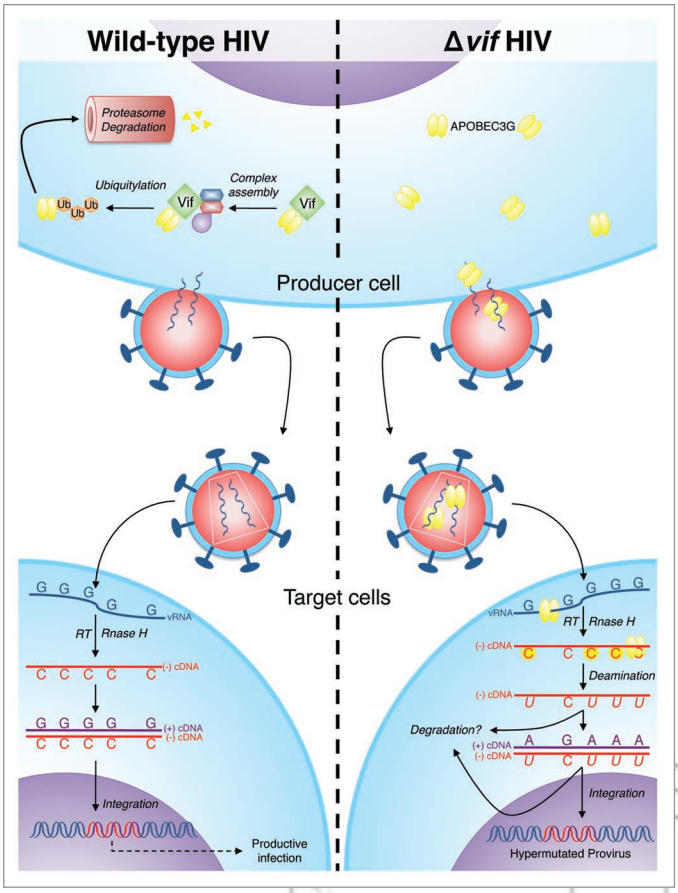


**Figure 2.** APOBEC3G restricts HIV infection. In the context of wild-type HIV infection, HIV Vif binds cellular APOBEC3G and recruits a cullin5-elongin B/C-Rbx ubiquitin ligase complex. APOBEC3G is polyubiquitylated and targeted for degradation in the proteasome. In the absence of HIV Vif, intact APOBEC3G packages with nascent virus particles and is delivered to newly infected cells. Upon reverse transcription, APOBEC3G edits newly synthesized (-) strand viral cDNA. Edited retrotranscripts are degraded or integrated as hypermutated proviruses.

for the identification of additional cytidine deaminase family members. Key features of AID/APOBEC1 domain organizations led to the bioinformatic identification of the primate APOBEC3 subfamily of cytidine deaminases.[29] These genes, designated APOBEC3A-3H, exhibit several characteristic family features, including zinc binding, at least one active site cluster, a linker region and a pseudoactive site domain. Upon their identification, the physiological role of these novel cytidine deaminases was unclear. However, initially unrelated work in HIV biology soon revealed the potent effects of the APOBEC3 enzymes on retroviral infection.

As a complex lentivirus, HIV is built from a genome containing the fundamental retroviral genes *gag*, *pol* and *env*, as well as several accessory genes that enhance viral infectivity.[30] Most of these accessory genes were successfully characterized relatively soon after their identification. However, a mechanistic function for virion infectivity factor (Vif) remained elusive. It was observed that Δ*vif* HIV infection is essentially unhindered in certain cell lines (so-called "permissive cells") yet dramatically diminished in other cell lines ("non-permissive cells") that otherwise support productive infection with wildtype HIV.[31,32] Heterokaryon studies suggested that the non-permissive cells expressed an endogenous viral restriction factor that was apparently obstructed by Vif.[33,34] A subtractive cDNA screen performed by Sheehy and colleagues identified this cellular restriction factor as CEM15,[35] also known as APOBEC3G.

APOBEC3G's place in the cytidine deaminase family provided clues as to how it might function to restrict Δ*vif* HIV infection. A torrent of work from various groups demonstrated that APOBEC3G restricts HIV by inducing hypermutation in newly reverse-transcribed viral cDNA (**Fig. 2**).[36,37] In non-permissive cells infected with Δ*vif* HIV, APOBEC3G associates with viral RNA[38,39] and/or the nucleocapsid domain of Gag,[40] which package the enzyme into newly assembled virions. Nascent viral particles then carry and deliver APOBEC3G to newly infected cells, in which the cytidine deaminase remains associated with the retroviral replication machinery. Upon reverse transcription and following activation by retroviral RNAseH activity,[41] APOBEC3G actively deaminates along the retroviral (-)-strand cDNA, introducing C-U changes through the viral retrogenome. Most hypermutated (-)-strand cDNAs do not proceed to second-strand synthesis, though the mechanism for their degradation remains unclear. Those retrotranscripts that do progress to integration carry a heavy mutation burden that drastically compromises the information content of the retroviral genome and prevents the production of functional virus.

Both $CD4^+$ T cells and macrophages, the principle targets of HIV infection in vivo, express high levels of APOBEC3G.[42] HIV establishes productive infections in these cell types through the action of Vif, which excludes APOBEC3G from packaging virions primarily by mediating its degradation.[43-47] In the infected cell, Vif binds APOBEG3G and recruits a cullin5-elongin B/C-Rbx ubiquitin ligase complex.[48,49] APOBEC3G is thereby targeted for ubiquitylation and subsequent degradation in the proteasome. This process allows for the production of virions unfettered by packaged APOBEC3G and corresponding hypermutation. The Vif/APOBEC3G interaction is precise and species-specific; HIV Vif inhibits APOBEC3G from human but not African Green Monkey (AGM), while SIV-AGM Vif inhibits AGM APOBEC3G, but not human.[50] This specificity maps to a single amino acid at position 128 in APOBEC3G (Asp in human, Lys in AGM).[51-53] Such exacting requirements for association offer a perspective on the powerful selective pressures exhibited by this host-pathogen relationship. In fact, phylogenetic analysis demonstrates that the APOBEC3 subfamily has been under markedly strong positive selection throughout primate evolution.[54,55] At present, it appears that the sole function of Vif is to act as a viral countermeasure against APOBEC3G and related family members. The evolutionary pressure to devote such a significant portion of the remarkably efficient HIV genome to this purpose illustrates the considerable impact this gene family can have on host defense.

**APOBEC3 family cytidine deaminases have diverse antiviral activities.** The antiviral activity of the polynucleotide cytidine deaminases is not limited to APOBEC3G, nor is it directed

solely against HIV. Aside from APOBEC3G, additional family members APOBEC3B and APOBEC3F can also restrict HIV replication (reviewed in ref. 56). Interestingly, APOBEC3B cannot be suppressed by Vif.[57,58] However, this may not be relevant in vivo, as only APOBEC3G and 3F are expressed by $CD4^+$ T cells and macrophages. Indeed, viral sequence analysis of HIV patient isolates reveals mutation patterns consistent with APOBEC3G and 3F activity.[59]

Apart from HIV, it seems that most retroviruses are somewhat susceptible to members of the APOBEC3 subfamily; Murine leukemia virus (MLV), Equine infectious anemia virus (EAIV), foamy retroviruses and even hepatitis B virus (HBV) can be restricted by different APOBEC3 deaminases (reviewed in ref. 60). Though not a true retrovirus, HBV replicates its partially double-stranded DNA genome by a reverse transcription mechanism,[61] which represents a likely target for these deaminases. However, reverse transcription may not be a requirement for susceptibility to all APOBEC3 subfamily members; APOBEC3A can dramatically inhibit replication of adeno-associated virus (AAV), a small single-stranded DNA parvovirus that replicates via the host cell polymerase machinery.[62] It remains to be seen if AAV represents a unique exception or if APOBEC3 subfamily members exhibit broad antiviral activity against diverse non-retroviruses.

**Endogenous retroelements are suppressed by APOBEC3 family cytidine deaminases.** The suppressive activity of the APOBEC3 proteins is not limited to exogenous retroviruses; several family members have also been demonstrated to restrict a variety of endogeneous retroelements. Such host-encoded retroelements constitute significant portions of mammalian genomes and include the long terminal repeat (LTR)-containing endogenous retroviruses (ERVs), as well as non-LTR sequences such as long interspersed nuclear element-1(LINE-1). Many of the APOBEC3 deaminases can suppress retrotransposition of ERVs[63] and (LINE-1) elements.[64] Though it remains unclear if the precise mechanism by which APOBEC3 proteins inhibit endogenous retroelements is identical to that employed in exogenous viral restriction, both appear to be significant targets for this defense system.

As the existence of functionally active ERVs in humans remains uncertain, the physiological relevance of APOBEC3 subfamily activity against these host-encoded sequences in vivo is not immediately apparent. Perhaps retroelement inhibition represents an "original" function of the APOBEC3 proteins; activity against exogenous lentiviruses may have been co-opted later in the evolutionary history of this gene family. Indeed, despite dramatic rates of positive selection throughout primate evolution, the apparent selective pressures on the APOBEC3 genes predate the emergence of modern primate lentiviruses by millions of years.[54,55] In addition, the evolutionary expansion of the APOBEC3 family correlates with a dramatic decrease of endogenous retroelement activity in primate genomes as compared to rodents.[65] Thus, the APOBEC3 proteins may represent an ancient defense system for protecting genome integrity, be it from actively mobile endogenous retroelements or the primitive retroviruses from which they were derived.

## Adenosine Deaminases Acting on RNA (ADARs) in the Immune System

**ADARs bind and edit dsRNA.** The adenosine deaminases acting on RNA (ADARs) bind to dsRNA structures and catalyze the conversion of adenosine to inosine (reviewed in refs. 66–69). Inosine is read as guanosine by translational and reverse transcriptional machinery. Thus, ADARs effectively induce A to G changes and thereby increase transcriptome diversity. A to I editing is the most common editing event in higher eukaryotes and has been recently recognized to occur predominantly in RNA duplexes formed from inverted Alu or LINE repeats in the untranslated regions (UTRs) of primary mRNA transcripts.[70-72] The biological consequences of editing within these sequence repeats are still uncertain but may serve a role in gene regulation. In fact, dsRNA Alu pairs in 3'UTRs can suppress gene expression, an event which correlates with A to I editing and the nuclear retention of certain transcripts.[73]

The ADARs were first identified as dsRNA-unwinding enzymes in *Xenopus laevis*[74,75] but were redefined shortly thereafter as dsRNA-editing enzymes.[76,77] Three ADARs (ADAR1-3) have been identified, based on conservation in the C-terminal deaminase and double-stranded RNA-binding domains.[69] ADAR1 and ADAR2 demonstrate deaminase activity[78-81] but the function of ADAR3 remains elusive.[82,83] ADAR1 is the best-characterized member of this gene family. Specifically, two differentially localized isoforms of ADAR1 have been identified. The larger form, ADAR1-L or ADAR p150, is present in both the cytosol and nucleus and is regulated by an interferon-inducible promoter.[84-86] ADAR1-S or ADAR1 p110, the smaller form, is exclusive to the nucleus and driven by two constitutively active promoters.[87]

ADARs are highly expressed in brain tissue and are important for neurological function. Site-specific ADAR editing in the brain, notably in glutamate and serotonin receptor mRNAs, leads to single codon changes and thereby protein products with altered physiological functions (reviewed in refs. 66, 67 and 69). While many of these neurological targets are reasonably well characterized, roles for ADARs in the immune system are presently under extensive investigation. In particular, ADAR1 has been implicated in hematopoeisis, viral immunity and regulation of the interferon response.

**ADAR1 is essential for immune cell development.** Multiple lines of evidence demonstrate the importance of A to I mRNA editing in the immune system, starting with a requirement for ADAR1 in immune cell development. More specifically, knockout studies have defined a role for ADAR1 in fetal hematopoeisis. ADAR1-deficient mice are known to suffer from liver and bone marrow hematopoietic defects and typically die at embryonic day 11.5–12.5.[88] Using ADAR1 conditional knock-out models, Orkin and colleagues have further demonstrated that ADAR1 is necessary for the maintenance of both fetal liver-derived and adult bone marrow hematopoietic stem cells (HSCs).[89] The authors propose that ADAR1 maintains HSC populations by protecting cells from early apoptotic events, possibly by regulating interferon signaling pathways (see below). Other recent work, however, suggests that ADAR1 activity is essential in the

differentiation of hematopoietic progenitor cells (HPCs), rather than HSCs.[90] Although the question remains as to which stage of hematopoiesis requires ADAR1, it is clear ADAR1-deficient precursors do not develop into mature immune cells.

**ADARs can act as antiviral enzymes.** As an interferon-inducible gene, it is not surprising that ADAR1 has been implicated in antiviral defense. As described above, it appears to be a critical regulator of the interferon response, which implies activity on host RNA targets. However, as an RNA editing enzyme, ADAR1 could presumably edit viral RNAs in a process not unlike APOBEC3 hypermutation of retroviral cDNA. Indeed, like HIV antagonism of APOBEC3G by the Vif protein, vaccinia virus and adenovirus have evolved ADAR1 inhibitors that specifically impair ADAR1 deaminase activity,[91,92] suggesting an anti-viral function for ADAR1. ADAR1 does edit a broad spectrum of viral targets but paradoxically seems to play both pro- and anti-viral roles in infection.

A to I editing has been observed in diverse viral RNAs, including influenza virus,[93] parainfluenza virus,[94] lymphocytic choriomeningitis virus (LCMV),[95] vesicular stomatitis virus (VSV),[96] measles virus,[106-108] polyomavirus,[97] hepatitis D virus (HDV)[98] and hepatitis C virus (HCV).[99] Despite an early recognition of A to I biased hyperediting in viral transcripts during persistent and lytic infections,[100] the function and consequences of many of these editing events is still under investigation. A clear example of direct ADAR antiviral editing has been observed in LCMV RNA transcripts.[95] In vitro and in vivo studies of LCMV infection demonstrated high rates of ADAR1-specific A-to-I mutations, leading to dysfunctional glycoproteins and impaired viral infectivity. In addition, recent work on HCV infection identified an ADAR1 editing-dependent loss of HCV replicons.[99] Replicon loss was thought to be attributable to an inosine-specific RNase[101,102] or to viral genome instability introduced by weakly base-pairing inosine nucleotides.[99] Another study using a polyoma virus system suggests that promiscuous A to I editing of transcripts in the nucleus may recruit an inosine-specific RNA binding protein, p54nrb. Along with the splicing factor PSF and the matrix structural protein matrin 3, binding of p54nrb functions to retain edited transcripts in the nucleus, preventing export and translation.[73,103] The authors suggest that this mechanism regulates the export of early stage transcripts, though it is possible that retention functions to sequester viral transcripts, inhibiting translation of essential viral proteins.

ADAR family members can also directly restrict viral replication independent of its editing function. ADAR1 associates with and activates transcription factors involved in anti-viral gene expression, including nuclear factor 90 (NF90).[104] ADAR1 interacts with NF90 via an undefined dsRNA bridge and leads to the upregulation of NF90-regulated genes, including IFNβ.

**ADARs can support viral infection.** In apparent contradiction to several well-characterized anti-viral functions, ADAR proteins can also promote viral infection and replication.

While p110 ADAR1 is the predominant isoform during embryogenesis,[105] the p150 interferon-inducible form is more prevalent in hematopoietic stem cells of the adult.[89] Expanding on their work with the conditional knockout mice, Orkin and colleagues showed that ADAR1 acts as novel suppressor of the Type I interferon response. Specifically, gene signatures of uninfected ADAR1-deficient HSCs and erythroid precursors are highly similar to those of virus-infected or interferon treated cells. Additionally, ADAR1 knockout embryos were found to have significantly higher levels of type 1 interferon in extracellular fluid. It remains to be explained how ADAR1 dampens the interferon response in the absence of viral infection. ADAR1 could be editing a microRNA molecule or target, neutralizing an unidentified immunostimulatory dsRNA or functioning in the regulation of interferon-induced gene expression. A compelling argument is that ADAR1 functions as a novel cytosolic dsRNA binding protein competing for substrates with the DNA-dependent activator of interferon regulatory factors (DAI), a cellular dsRNA sensor. In sequestering immunostimulatory dsRNA (e.g., viral replication intermediates) from DAI, ADAR1 may impair downstream innate immune signaling.[106] The absence of ADAR1 dsRNA binding activity could lead to aberrant activation of the innate immune response and a corresponding induction of interferon production.

Perhaps related to its role in regulation of the interferon response, ADAR1 has been observed to bind and impair host antiviral response elements. Work on measles viral infection has shown ADAR1 inhibition of protein kinase regulated by RNA (PKR) and interferon regulatory transcription factor-3 (IRF-3).[107] Other groups have observed similar ADAR1 regulation of PKR during VSV[108,109] and HIV (see below) infections as well. Inhibition of these proteins compromises the host cell ability to respond to viral signals, potentially promoting persistent infection. Interestingly, hyperediting of viral transcripts has been observed in patients suffering from a complication of persistent measles infection. This paradoxical situation, in which there is evidence of ADAR1 activity both supporting and antagonizing viral infection suggests a nuanced role for ADAR1 in host-virus interaction.

It appears that ADAR1 can dampen type I interferon signaling by several mechanisms. This function may have evolved to protect the host from an inappropriate (i.e., in uninfected cells) or overactive (i.e., disproportionate response to infection) interferon response. This regulation might serve to counterbalance the effector functions of ADAR1 as an antiviral enzyme. However, it seems that in certain infections, ADAR1 anti-viral activity is not only impaired but the enzyme itself may be co-opted by viruses to support infection. In this context, the significance of ADAR1 hyperediting viral RNA remains unclear. Is it merely an inert footprint of ADAR1 activity or do some editing events still function to restrict (or promote) viral replication? Additional studies, particularly using the recently engineered conditional ADAR1-deficient mouse will likely address these problems.

**ADAR1 acts in the host response to HIV infection.** Like its deaminase cousin APOBEC3G, ADAR1 has recently been found to target and edit HIV-1 sequences. However, unlike APOBEC3G, which edits retrotranscript cDNA and restricts viral infection, ADAR1 targets viral RNA and enhances HIV protein expression, replication and infectivity.[110] These effects are mediated by both editing-dependent and editing-independent

mechanisms. Overexpression of ADAR1 in HIV-1 producer cells dramatically enhances expression of several viral proteins irrespective of RNA editing, possibly due to ADAR inhibition of PKR.[111] Active RNA editing may also regulate virus production as ADAR1, but not experimentally-engineered catalytic mutants, increased the release of progeny virions 2-fold and enhanced HIV-1 infectivity 2.5-fold.[110]

The physiological significance of ADAR1 action on the HIV-1 life cycle is still unclear, as are the exact viral sequence targets for ADAR1 editing. One analysis found that a majority of A to G changes occur in a region of the 5'UTR shared by all HIV-1 transcripts.[110] A different study identified a specific ADAR1 editing site in the HIV env gene.[112] Further investigation is needed to understand the regulation, impact and mechanism of ADAR1 editing in HIV-1 RNA. Of particular interest are the opposing roles of the cytidine (APOBEC3G) and adenosine (ADAR1) deaminases in HIV-1 infection. While APOBEC3G targets and edits virion RNA to restrict and inhibit HIV-1, ADAR1 appears to be harnessed by the virus itself to enhance viral protein expression, infectivity and virion release.

**ADAR1 edits hepatitis D viral RNA.** The best characterized ADAR-mediated viral editing event occurs during HDV infection. HDV is a subviral pathogen that is dependent on a concurrent infection with HBV; it requires HBV surface antigen to infect hepatocytes.[113,114] The genome of the HDV virus is an ideal substrate for ADARs, as its single-stranded negative sense circular RNA forms secondary structures with frequent duplex regions.[115] In addition to co-opting the hepatitis B surface antigen, the HDV genome encodes its own HDV-specific surface antigen (HDVAg), the antigenome transcript of which is edited by ADAR1 in a site-specific manner.[98,116,117] The HDVAg occurs in two forms, both essential for the viral life cycle. The short form, HDVAg-S is required for viral RNA replication[118] while the long form (HDVAg-L) directs viral genome assembly and packaging.[119,120] ADAR1 targets the HDV antigenome at a specific "amber/W site," thereby converting a stop codon (UAG) to a tryptophan (UIG). This allows for the translation of the long form of the HDV surface antigen.[98,116,117] The HDVAg-L then restricts viral replication in a trans-dominant fashion by binding HDAg-S and interrupting HDAg-S homodimers.[121,122]

In unstimulated cells, amber/W site editing occurs via the ADAR1-S isoform[123,124] and serves to support viral assembly; HDVAg-L halts replication and mediates viral packaging by binding clathrin heavy chain.[125] More recent studies have shown, however, that ADAR1 can serve an anti-viral role when editing in a more promiscuous fashion. Overexpression of ADAR1 or ADAR2 (though ADAR2 is not induced in natural infection) leads to hyperediting at non-amber/W sites, producing higher levels of HDAg-L as well as other HDAg mutants that can also bind HDAg-S and inhibit replication. In an interferon-stimulated system, which more closely mimics both early natural infection[126] and IFN-treated infection, the large form of ADAR1 is highly expressed, is the predominant editor, and increases editing 2-fold.[127] It is unclear, however, whether IFN stimulation would induce high enough levels of ADAR1-L to edit promiscuously or to significantly impair viral replication. In fact, while a replication-competent mutant virus with enhanced editing at the amber/W site displays increased levels of HDAg-L early in infection and impaired replication at a later time point, editing and replication seem to be coupled.[128] Amber/W site editing ceases with replication, indicating a natural feedback mechanism controlling aberrant editing by elevated ADAR1 activity.

## Closing Remarks

Polynucleotide deaminases function as drivers for diversity by altering the information content of RNA and DNA sequences. Here, we have described some of the processes by which this family of enzymes function in the host response to pathogenic challenges. The polynucleotide deaminases act on a variety of molecular substrates (including ssRNA, dsRNA and ssDNA), catalyze different editing events (C to U; A to I), edit a diverse set of sequence targets, and not surprisingly, play a number of roles in innate and adaptive immunity. Significantly, the immune functions of these editing enzymes manifest on both sides of the host:pathogen interface. AID drives the plasticity of the Ig locus and thereby mediates key features of the adaptive immune response. APOBEC3 and ADAR proteins are potent innate immune effectors and can incapacitate viral genomes by directing their mutagenic activity against viral sequences. Paradoxically, ADAR1 can also promote viral replication and/or diversification by acting on both pathogen and host targets. Though the past decade of research has vastly increased our understanding of these enzyme families, especially with regard to immune function, many unanswered questions remain. In particular, there are likely roles for RNA/DNA editing in additional host defense mechanisms. The identification and functional characterization of novel APOBEC and ADAR targets may reveal important novel functions for these enzymes in the immune system.



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## References


1. Navaratnam N, Morrison JR, Bhattacharya S, Patel D, Funahashi T, Giannoni F, et al. The p27 catalytic subunit of the apolipoprotein B mRNA editing enzyme is a cytidine deaminase. J Biol Chem 1993; 268:20709-12.
2. Teng B, Burant CF, Davidson NO. Molecular cloning of an apolipoprotein B messenger RNA editing protein. Science 1993; 260:1816-9.
3. Mehta A, Kinter MT, Sherman NE, Driscoll DM. Molecular cloning of apobec-1 complementation factor, a novel RNA-binding protein involved in the editing of apolipoprotein B mRNA. Mol Cell Biol 2000; 20:1846-54.
4. Chester A, Somasekaram A, Tzimina M, Jarmuz A, Gisbourne J, O'Keefe R, et al. The apolipoprotein B mRNA editing complex performs a multifunctional cycle and suppresses nonsense-mediated decay. EMBO J 2003; 22:3971-82.
5. Anant S, Davidson NO. An AU-rich sequence element (UUUN[A/U]U) downstream of the edited C in apolipoprotein B mRNA is a high-affinity binding site for Apobec-1: binding of Apobec-1 to this motif in the 3' untranslated region of c-myc increases mRNA stability. Mol Cell Biol 2000; 20:1982-92.
6. Anant S, Murmu N, Houchen CW, Mukhopadhyay D, Riehl TE, Young SG, et al. Apobec-1 protects intestine from radiation injury through posttranscriptional regulation of cyclooxygenase-2 expression. Gastroenterology 2004; 127:1139-49.
7. Xie Y, Blanc V, Kerr TA, Kennedy S, Luo J, Newberry EP, et al. Decreased Expression of Cholesterol 7{alpha}-Hydroxylase and Altered Bile Acid Metabolism in Apobec-1$^{-/-}$ Mice Lead to Increased Gallstone Susceptibility. J Biol Chem 2009; 284:16860-71.
8. Nakamuta M, Oka K, Krushkal J, Kobayashi K, Yamamoto M, Li WH, et al. Alternative mRNA splicing and differential promoter utilization determine tissue-specific expression of the apolipoprotein B mRNA-editing protein (Apobec1) gene in mice. Structure and evolution of Apobec1 and related nucleoside/nucleotide deaminases. J Biol Chem 1995; 270:13042-56.
9. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell 2000; 102:553-63.
10. Muramatsu M, Sankaranand VS, Anant S, Sugai M, Kinoshita K, Davidson NO, et al. Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. J Biol Chem 1999; 274:18470-6.
11. Neuberger MS, Ehrenstein MR, Klix N, Jolly CJ, Yelamos J, Rada C, et al. Monitoring and interpreting the intrinsic features of somatic hypermutation. Immunol Rev 1998; 162:107-16.
12. Peters A, Storb U. Somatic hypermutation of immunoglobulin genes is linked to transcription initiation. Immunity 1996; 4:57-65.
13. Wiesendanger M, Scharff MD, Edelmann W. Somatic hypermutation, transcription and DNA mismatch repair. Cell 1998; 94:415-8.
14. Martin A, Scharff MD. Somatic hypermutation of the AID transgene in B and non-B cells. Proc Natl Acad Sci USA 2002; 99:12304-8.
15. Yoshikawa K, Okazaki I-M, Eto T, Kinoshita K, Muramatsu M, Nagaoka H, et al. AID enzyme-induced hypermutation in an actively transcribed gene in fibroblasts. Science 2002; 296:2033-6.
16. Mayorov VI, Rogozin IB, Adkison LR, Frahm C, Kunkel TA, Pavlov YI. Expression of human AID in yeast induces mutations in context similar to the context of somatic hypermutation at G-C pairs in immunoglobulin genes. BMC Immunol 2005; 6:10.
17. Petersen-Mahrt SK, Harris RS, Neuberger MS. AID mutates *E. coli* suggesting a DNA deamination mechanism for antibody diversification. Nature 2002; 418:99-103.
18. Rada C, Williams GT, Nilsen H, Barnes DE, Lindahl T, Neuberger MS. Immunoglobulin isotype switching is inhibited and somatic hypermutation perturbed in UNG-deficient mice. Curr Biol 2002; 12:1748-55.
19. Liu M, Duke JL, Richter DJ, Vinuesa CG, Goodnow CC, Kleinstein SH, et al. Two levels of protection for the B cell genome during somatic hypermutation. Nature 2008; 451:841-5.
20. Robbiani DF, Bothmer A, Callen E, Reina-San-Martin B, Dorsett Y, Difilippantonio S, et al. AID is required for the chromosomal breaks in c-myc that lead to c-myc/IgH translocations. Cell 2008; 135:1028-38.
21. Raghavan SC, Swanson PC, Wu X, Hsieh C-L, Lieber MR. A non-B-DNA structure at the Bcl-2 major breakpoint region is cleaved by the RAG complex. Nature 2004; 428:88-93.
22. Ramiro AR, Jankovic M, Callen E, Difilippantonio S, Chen HT, McBride KM, et al. Role of genomic instability and p53 in AID-induced c-myc-Igh translocations. Nature 2006; 440:105-9.
23. Wakae K, Magor BG, Saunders H, Nagaoka H, Kawamura A, Kinoshita K, et al. Evolution of class switch recombination function in fish activation-induced cytidine deaminase, AID. Int Immunol 2006; 18:41-7.
24. Gourzi P, Leonova T, Papavasiliou FN. A role for activation-induced cytidine deaminase in the host response against a transforming retrovirus. Immunity 2006; 24:779-86.
25. Lin C, Yang L, Tanasa B, Hutt K, Ju B-g, Ohgi K, et al. Nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific translocations in cancer. Cell 2009; 139:1069-83.
26. Chiba T, Marusawa H. A novel mechanism for inflammation-associated carcinogenesis: an important role of activation-induced cytidine deaminase (AID) in mutation induction. J Mol Med 2009; 87:1023-7.
27. Bhutani N, Brady JJ, Damian M, Sacco A, Corbel SY, Blau HM. Reprogramming towards pluripotency requires AID-dependent DNA demethylation. Nature 2009.
28. Feldhahn N, Henke N, Melchior K, Duy C, Soh BN, Klein F, et al. Activation-induced cytidine deaminase acts as a mutator in BCR-ABL1-transformed acute lymphoblastic leukemia cells. J Exp Med 2007; 204:1157-66.
29. Jarmuz A, Chester A, Bayliss J, Gisbourne J, Dunham I, Scott J, et al. An anthropoid-specific locus of orphan C to U RNA-editing enzymes on chromosome 22. Genomics 2002; 79:285-96.
30. Malim MH, Emerman M. HIV-1 accessory proteins—ensuring viral survival in a hostile environment. Cell Host Microbe 2008; 3:388-98.
31. Gabuzda DH, Lawrence K, Langhoff E, Terwilliger E, Dorfman T, Haseltine WA, et al. Role of vif in replication of human immunodeficiency virus type 1 in CD4$^+$ T lymphocytes. J Virol 1992; 66:6489-95.
32. von Schwedler U, Song J, Aiken C, Trono D. Vif is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells. J Virol 1993; 67:4945-55.
33. Madani N, Kabat D. An endogenous inhibitor of human immunodeficiency virus in human lymphocytes is overcome by the viral Vif protein. J Virol 1998; 72:10251-5.
34. Simon JH, Gaddis NC, Fouchier RA, Malim MH. Evidence for a newly discovered cellular anti-HIV-1 phenotype. Nat Med 1998; 4:1397-400.
35. Sheehy AM, Gaddis NC, Choi JD, Malim MH. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature 2002; 418:646-50.
36. Harris RS, Bishop KN, Sheehy AM, Craig HM, Petersen-Mahrt SK, Watt IN, et al. DNA deamination mediates innate immunity to retroviral infection. Cell 2003; 113:803-9.
37. Lecossier D, Bouchonnet F, Clavel F, Hance AJ. Hypermutation of HIV-1 DNA in the absence of the Vif protein. Science 2003; 300:1112.
38. Khan MA, Kao S, Miyagi E, Takeuchi H, Goila-Gaur R, Opi S, et al. Viral RNA is required for the association of APOBEC3G with human immunodeficiency virus type 1 nucleoprotein complexes. J Virol 2005; 79:5870-4.
39. Svarovskaia ES, Xu H, Mbisa JL, Barr R, Gorelick RJ, Ono A, et al. 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 2004; 279:35822-8.
40. Schäfer A, Bogerd HP, Cullen BR. Specific packaging of APOBEC3G into HIV-1 virions is mediated by the nucleocapsid domain of the gag polyprotein precursor. Virology 2004; 328:163-8.
41. Soros VB, Yonemoto W, Greene WC. Newly synthesized APOBEC3G is incorporated into HIV virions, inhibited by HIV RNA, and subsequently activated by RNase H. PLoS Pathog 2007; 3:15.
42. Chiu Y-L, Soros VB, Kreisberg JF, Stopak K, Yonemoto W, Greene WC. Cellular APOBEC3G restricts HIV-1 infection in resting CD4$^+$ T cells. Nature 2005; 435:108-14.
43. Conticello SG, Harris RS, Neuberger MS. The Vif protein of HIV triggers degradation of the human antiretroviral DNA deaminase APOBEC3G. Curr Biol 2003; 13:2009-13.
44. Marin M, Rose KM, Kozak SL, Kabat D. HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. Nat Med 2003; 9:1398-403.
45. Mehle A, Strack B, Ancuta P, Zhang C, McPike M, Gabuzda D. Vif overcomes the innate antiviral activity of APOBEC3G by promoting its degradation in the ubiquitin-proteasome pathway. J Biol Chem 2004; 279:7792-8.
46. Sheehy AM, Gaddis NC, Malim MH. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. Nat Med 2003; 9:1404-7.
47. Stopak K, de Noronha C, Yonemoto W, Greene WC. HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. Mol Cell 2003; 12:591-601.
48. Kobayashi M, Takaori-Kondo A, Miyauchi Y, Iwai K, Uchiyama T. Ubiquitination of APOBEC3G by an HIV-1 Vif-Cullin5-Elongin B-Elongin C complex is essential for Vif function. J Biol Chem 2005; 280:18573-8.
49. Yu X, Yu Y, Liu B, Luo K, Kong W, Mao P, et al. Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. Science 2003; 302:1056-60.
50. Mariani R, Chen D, Schröfelbauer B, Navarro F, König R, Bollman B, et al. Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. Cell 2003; 114:21-31.
51. Bogerd HP, Doehle BP, Wiegand HL, Cullen BR. 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 USA 2004; 101:3770-4.
52. Mangeat B, Turelli P, Liao S, Trono D. A single amino acid determinant governs the species-specific sensitivity of APOBEC3G to Vif action. J Biol Chem 2004; 279:14481-3.
53. Schröfelbauer B, Senger T, Manning G, Landau NR. Mutational alteration of human immunodeficiency virus type 1 Vif allows for functional interaction with nonhuman primate APOBEC3G. J Virol 2006; 80:5984-91.
54. Sawyer SL, Emerman M, Malik HS. Ancient adaptive evolution of the primate antiviral DNA-editing enzyme APOBEC3G. PLoS Biol 2004; 2:275.
55. Zhang J, Webb DM. Rapid evolution of primate antiviral enzyme APOBEC3G. Hum Mol Genet 2004; 13:1785-91.

56. Turelli P, Trono D. Editing at the crossroad of innate and adaptive immunity. Science 2005; 307:1061-5.
57. Bishop KN, Holmes RK, Sheehy AM, Davidson NO, Cho S-J, Malim MH. Cytidine deamination of retroviral DNA by diverse APOBEC proteins. Curr Biol 2004; 14:1392-6.
58. Doehle BP, Schäfer A, Cullen BR. Human APOBEC3B is a potent inhibitor of HIV-1 infectivity and is resistant to HIV-1 Vif. Virology 2005; 339:281-8.
59. Simon V, Zennou V, Murray D, Huang Y, Ho DD, Bieniasz PD. Natural variation in Vif: differential impact on APOBEC3G/3F and a potential role in HIV-1 diversification. PLoS Pathog 2005; 1:6.
60. Rosenberg BR, Papavasiliou FN. Beyond SHM and CSR: AID and related cytidine deaminases in the host response to viral infection. Adv Immunol 2007; 94:215-44.
61. Ganem D. Hepadnaviridae and their replication. In: Fields B, Knipe D, Howley P, eds. Fields Virology. Philadelphia: Lippincott-Raven 1996; 2703-37.
62. Chen H, Lilley CE, Yu Q, Lee DV, Chou J, Narvaiza I, et al. APOBEC3A is a potent inhibitor of adeno-associated virus and retrotransposons. Curr Biol 2006; 16:480-5.
63. Esnault C, Heidmann O, Delebecque F, Dewannieux M, Ribet D, Hance AJ, et al. APOBEC3G cytidine deaminase inhibits retrotransposition of endogenous retroviruses. Nature 2005; 433:430-3.
64. Muckenfuss H, Hamdorf M, Held U, Perkovic M, Löwer J, Cichutek K, et al. APOBEC3 proteins inhibit human LINE-1 retrotransposition. J Biol Chem 2006; 281:22161-72.
65. Consortium MGS, Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, et al. Initial sequencing and comparative analysis of the mouse genome. Nature 2002; 420:520-62.
66. Bass BL. RNA editing by adenosine deaminases that act on RNA. Annu Rev Biochem 2002; 71:817-46.
67. Keegan LP, Leroy A, Sproul D, O'Connell MA. Adenosine deaminases acting on RNA (ADARs): RNA-editing enzymes. Genome Biol 2004; 5:209.
68. Nishikura K. Editor meets silencer: crosstalk between RNA editing and RNA interference. Nat Rev Mol Cell Biol 2006; 7:919-31.
69. Valente L, Nishikura K. ADAR gene family and A-to-I RNA editing: diverse roles in posttranscriptional gene regulation. Prog Nucleic Acid Res Mol Biol 2005; 79:299-338.
70. Athanasiadis A, Rich A, Maas S. Widespread A-to-I RNA editing of Alu-containing mRNAs in the human transcriptome. PLoS Biol 2004; 2:391.
71. Kim DDY, Kim TTY, Walsh T, Kobayashi Y, Matise TC, Buyske S, et al. Widespread RNA editing of embedded alu elements in the human transcriptome. Genome Res 2004; 14:1719-25.
72. Levanon EY, Eisenberg E, Yelin R, Nemzer S, Hallegger M, Shemesh R, et al. Systematic identification of abundant A-to-I editing sites in the human transcriptome. Nat Biotechnol 2004; 22:1001-5.
73. Chen L-L, DeCerbo JN, Carmichael GG. Alu element-mediated gene silencing. EMBO J 2008; 27:1694-705.
74. Bass BL, Weintraub H. A developmentally regulated activity that unwinds RNA duplexes. Cell 1987; 48:607-13.
75. Rebagliati MR, Melton DA. Antisense RNA injections in fertilized frog eggs reveal an RNA duplex unwinding activity. Cell 1987; 48:599-605.
76. Bass BL, Weintraub H. An unwinding activity that covalently modifies its double-stranded RNA substrate. Cell 1988; 55:1089-98.
77. Wagner RW, Smith JE, Cooperman BS, Nishikura K. A double-stranded RNA unwinding activity introduces structural alterations by means of adenosine to inosine conversions in mammalian cells and Xenopus eggs. Proc Natl Acad Sci USA 1989; 86:2647-51.
78. Gerber A, O'Connell MA, Keller W. Two forms of human double-stranded RNA-specific editase 1 (hRED1) generated by the insertion of an Alu cassette. RNA 1997; 3:453-63.
79. Kim U, Wang Y, Sanford T, Zeng Y, Nishikura K. Molecular cloning of cDNA for double-stranded RNA adenosine deaminase, a candidate enzyme for nuclear RNA editing. Proc Natl Acad Sci USA 1994; 91:11457-61.
80. Lai F, Chen CX, Carter KC, Nishikura K. Editing of glutamate receptor B subunit ion channel RNAs by four alternatively spliced DRADA2 double-stranded RNA adenosine deaminases. Mol Cell Biol 1997; 17:2413-24.
81. Melcher T, Maas S, Herb A, Sprengel R, Seeburg PH, Higuchi M. A mammalian RNA editing enzyme. Nature 1996; 379:460-4.
82. Chen CX, Cho DS, Wang Q, Lai F, Carter KC, Nishikura K. A third member of the RNA-specific adenosine deaminase gene family, ADAR3, contains both single- and double-stranded RNA binding domains. RNA 2000; 6:755-67.
83. Melcher T, Maas S, Herb A, Sprengel R, Higuchi M, Seeburg PH. RED2, a brain-specific member of the RNA-specific adenosine deaminase family. J Biol Chem 1996; 271:31795-8.
84. George CX, Samuel CE. Human RNA-specific adenosine deaminase ADAR1 transcripts possess alternative exon 1 structures that initiate from different promoters, one constitutively active and the other interferon inducible. Proc Natl Acad Sci USA 1999; 96:4621-6.
85. George CX, Samuel CE. Characterization of the 5'-flanking region of the human RNA-specific adenosine deaminase ADAR1 gene and identification of an interferon-inducible ADAR1 promoter. Gene 1999; 229:203-13.
86. Patterson JB, Samuel CE. Expression and regulation by interferon of a double-stranded-RNA-specific adenosine deaminase from human cells: evidence for two forms of the deaminase. Mol Cell Biol 1995; 15:5376-88.
87. Kawakubo K, Samuel CE. Human RNA-specific adenosine deaminase (ADAR1) gene specifies transcripts that initiate from a constitutively active alternative promoter. Gene 2000; 258:165-72.
88. Hartner JC, Schmittwolf C, Kispert A, Müller AM, Higuchi M, Seeburg PH. Liver disintegration in the mouse embryo caused by deficiency in the RNA-editing enzyme ADAR1. J Biol Chem 2004; 279:4894-902.
89. Hartner JC, Walkley CR, Lu J, Orkin SH. ADAR1 is essential for the maintenance of hematopoiesis and suppression of interferon signaling. Nat Immunol 2009; 10:109-15.
90. XuFeng R, Boyer MJ, Shen H, Li Y, Yu H, Gao Y, et al. ADAR1 is required for hematopoietic progenitor cell survival via RNA editing. Proc Natl Acad Sci USA 2009; 106:17763-8.
91. Lei M, Liu Y, Samuel CE. Adenovirus VAI RNA antagonizes the RNA-editing activity of the ADAR adenosine deaminase. Virology 1998; 245:188-96.
92. Liu Y, Wolff KC, Jacobs BL, Samuel CE. Vaccinia virus E3L interferon resistance protein inhibits the interferon-induced adenosine deaminase A-to-I editing activity. Virology 2001; 289:378-87.
93. Tenoever BR, Ng S-L, Chua MA, McWhirter SM, García-Sastre A, Maniatis T. Multiple functions of the IKK-related kinase IKKepsilon in interferon-mediated antiviral immunity. Science 2007; 315:1274-8.
94. Murphy DG, Dimock K, Kang CY. Numerous transitions in human parainfluenza virus 3 RNA recovered from persistently infected cells. Virology 1991; 181:760-3.
95. Zahn RC, Schelp I, Utermöhlen O, von Laer D. A-to-G hypermutation in the genome of lymphocytic choriomeningitis virus. J Virol 2007; 81:457-64.
96. O'Hara PJ, Nichol ST, Horodyski FM, Holland JJ. Vesicular stomatitis virus defective interfering particles can contain extensive genomic sequence rearrangements and base substitutions. Cell 1984; 36:915-24.
97. Kumar M, Carmichael GG. Nuclear antisense RNA induces extensive adenosine modifications and nuclear retention of target transcripts. Proc Natl Acad Sci USA 1997; 94:3542-7.
98. Luo GX, Chao M, Hsieh SY, Sureau C, Nishikura K, Taylor J. A specific base transition occurs on replicating hepatitis delta virus RNA. J Virol 1990; 64:1021-7.
99. Taylor DR, Puig M, Darnell MER, Mihalik K, Feinstone SM. New antiviral pathway that mediates hepatitis C virus replicon interferon sensitivity through ADAR1. J Virol 2005; 79:6291-8.
100. Cattaneo R. Biased (A→I) hypermutation of animal RNA virus genomes. Curr Opin Genet Dev 1994; 4:895-900.
101. Scadden AD, Smith CW. A ribonuclease specific for inosine-containing RNA: a potential role in antiviral defence? EMBO J 1997; 16:2140-9.
102. Scadden AD, Smith CW. RNAi is antagonized by A→I hyper-editing. EMBO Rep 2001; 2:1107-11.
103. Zhang Z, Carmichael GG. The fate of dsRNA in the nucleus: a p54(nrb)-containing complex mediates the nuclear retention of promiscuously A-to-I edited RNAs. Cell 2001; 106:465-75.
104. Nie Y, Ding L, Kao PN, Braun R, Yang J-H. ADAR1 interacts with NF90 through double-stranded RNA and regulates NF90-mediated gene expression independently of RNA editing. Mol Cell Biol 2005; 25:6956-63.
105. George CX, Wagner MV, Samuel CE. Expression of interferon-inducible RNA adenosine deaminase ADAR1 during pathogen infection and mouse embryo development involves tissue-selective promoter utilization and alternative splicing. J Biol Chem 2005; 280:15020-8.
106. Wang Z, Choi MK, Ban T, Yanai H, Negishi H, Lu Y, et al. Regulation of innate immune responses by DAI (DLM-1/ZBP1) and other DNA-sensing molecules. Proc Natl Acad Sci USA 2008; 105:5477-82.
107. Toth AM, Li Z, Cattaneo R, Samuel CE. RNA-specific adenosine deaminase ADAR1 suppresses measles virus-induced apoptosis and activation of protein kinase PKR. J Biol Chem 2009; 284:29350-6.
108. Li Z, Wolff K, Samuel C. RNA adenosine deaminase ADAR1 deficiency leads to increased activation of protein kinase PKR and reduced vesicular stomatitis virus growth following interferon treatment. Virology 2009.
109. Nie Y, Hammond GL, Yang J-H. Double-stranded RNA deaminase ADAR1 increases host susceptibility to virus infection. J Virol 2007; 81:917-23.
110. Doria M, Neri F, Gallo A, Farace MG, Michienzi A. Editing of HIV-1 RNA by the double-stranded RNA deaminase ADAR1 stimulates viral infection. Nucleic Acids Res 2009; 37:5848-58.
111. Clerzius G, Gélinas J-F, Daher A, Bonnet M, Meurs EF, Gatignol A. ADAR1 interacts with PKR during human immunodeficiency virus infection of lymphocytes and contributes to viral replication. J Virol 2009; 83:10119-28.
112. Phuphuakrat A, Kraiwong R, Boonarkart C, Lauhakirti D, Lee T-H, Auewarakul P. Double-stranded RNA adenosine deaminases enhance expression of human immunodeficiency virus type 1 proteins. J Virol 2008; 82:10864-72.
113. Lai MM. The molecular biology of hepatitis delta virus. Annu Rev Biochem 1995; 64:259-86.
114. Taylor JM. Replication of human hepatitis delta virus: recent developments. Trends Microbiol 2003; 11:185-90.
115. Wang KS, Choo QL, Weiner AJ, Ou JH, Najarian RC, Thayer RM, et al. Structure, sequence and expression of the hepatitis delta (delta) viral genome. Nature 1986; 323:508-14.

116. Casey JL, Gerin JL. Hepatitis D virus RNA editing: specific modification of adenosine in the antigenomic RNA. J Virol 1995; 69:7593-600.
117. Polson AG, Bass BL, Casey JL. RNA editing of hepatitis delta virus antigenome by dsRNA-adenosine deaminase. Nature 1996; 380:454-6.
118. Kuo MY, Chao M, Taylor J. Initiation of replication of the human hepatitis delta virus genome from cloned DNA: role of delta antigen. J Virol 1989; 63:1945-50.
119. Chang FL, Chen PJ, Tu SJ, Wang CJ, Chen DS. The large form of hepatitis delta antigen is crucial for assembly of hepatitis delta virus. Proc Natl Acad Sci USA 1991; 88:8490-4.
120. Ryu WS, Bayer M, Taylor J. Assembly of hepatitis delta virus particles. J Virol 1992; 66:2310-5.
121. Chao M, Hsieh SY, Taylor J. Role of two forms of hepatitis delta virus antigen: evidence for a mechanism of self-limiting genome replication. J Virol 1990; 64:5066-9.
122. Glenn JS, White JM. trans-dominant inhibition of human hepatitis delta virus genome replication. J Virol 1991; 65:2357-61.
123. Jayan GC, Casey JL. Inhibition of hepatitis delta virus RNA editing by short inhibitory RNA-mediated knockdown of ADAR1 but not ADAR2 expression. J Virol 2002; 76:12399-404.
124. Wong SK, Lazinski DW. Replicating hepatitis delta virus RNA is edited in the nucleus by the small form of ADAR1. Proc Natl Acad Sci USA 2002; 99:15118-23.
125. Huang C, Chang SC, Yang H-C, Chien C-L, Chang M-F. Clathrin-mediated post-Golgi membrane trafficking in the morphogenesis of hepatitis delta virus. J Virol 2009; 83:12314-24.
126. Hartwig D, Schoeneich L, Greeve J, Schütte C, Dorn I, Kirchner H, et al. Interferon-alpha stimulation of liver cells enhances hepatitis delta virus RNA editing in early infection. J Hepatol 2004; 41:667-72.
127. Hartwig D, Schütte C, Warnecke J, Dorn I, Hennig H, Kirchner H, et al. The large form of ADAR 1 is responsible for enhanced hepatitis delta virus RNA editing in interferon-alpha-stimulated host cells. J Viral Hepat 2006; 13:150-7.
128. Sato S, Cornillez-Ty C, Lazinski DW. By inhibiting replication, the large hepatitis delta antigen can indirectly regulate amber/W editing and its own expression. J Virol 2004; 78:8120-34.