ANNUAL REVIEWS

Annual Review of Virology

Adenosine Deaminases Acting on RNA (ADARs) and Viral Infections

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Annu. Rev. Virol. 2021. 8:239–64

First published as a Review in Advance on April 21, 2021

The *Annual Review of Virology* is online at virology.annualreviews.org

https://doi.org/10.1146/annurev-virology-091919-065320

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Keywords

adenosine deaminase acting on RNA, ADAR, autoimmunity, double-stranded RNA, dsRNA, innate immunity, interferon, RNA editing

Abstract

C6 deamination of adenosine (A) to inosine (I) in double-stranded RNA (dsRNA) is catalyzed by a family of enzymes known as ADARs (adenosine deaminases acting on RNA) encoded by three genes in mammals. Alternative promoters and splicing produce two ADAR1 proteins, an interferon-inducible cytoplasmic p150 and a constitutively expressed p110 that like ADAR2 is a nuclear enzyme. ADAR3 lacks deaminase activity. A-to-I editing occurs with both viral and cellular RNAs. Deamination activity is dependent on dsRNA substrate structure and regulatory RNA-binding proteins and ranges from highly site selective with hepatitis D RNA and glutamate receptor precursor messenger RNA (pre-mRNA) to hyperediting of measles virus and polyomavirus transcripts and cellular inverted *Alu* elements. Because I base-pairs as guanosine instead of A, editing can alter mRNA decoding, pre-mRNA splicing, and microRNA silencing. Editing also alters dsRNA structure, thereby suppressing innate immune responses including interferon production and action.

INTRODUCTION: ADARs AND THEIR REGULATION

Adenosine deaminases acting on RNA, known as ADARs (1), were discovered during antisense RNA studies in *Xenopus* (2, 3). Sense-antisense double-stranded (ds) RNA structures were destabilized based on their altered mobility during native gel electrophoresis and increased sensitivity to nuclease degradation by single-strand selective RNase. The activity responsible for these changes in dsRNA structure catalyzed the C6 deamination of adenosine (A), a covalent modification that produces inosine (I) (**Figure 1*a***). This process now is called A-to-I editing (4–8). Because I generated by ADAR base-pairs as guanosine (G) instead of A, the deamination effectively alters the RNA sequence by making a purine nucleotide substitution of I (= G) for A (**Figure 1*b***). Such substitutions can alter coding, as I base-pairs with cytosine (C) instead of uracil (U). Substitution of I for A also can alter RNA structure because an I-U mismatch pair is less stable than an A:U pair (9).

ADARs are different from adenosine deaminases (ADAs) that also catalyze the C6 deamination of A. The substrate for ADA is the free nucleoside A, whereas ADARs deaminate A present in dsRNA. ADA is an important enzyme of the purine salvage pathway. Loss of ADA activity results in severe combined immunodeficiency (10). Dysregulation of ADAR1 activity also results in human diseases including Aicardi-Goutières syndrome, an autoimmune disorder and interferonopathy (11); bilateral striatal necrosis dystonia (12); and dyschromatosis symmetrica hereditaria, a skin pigmentation disorder (13).

This review concerns the regulation and functions of ADARs and the roles they play during viral infections. dsRNA has a long history in virology and innate immunity, the cornerstone of which is the interferon (IFN) response (14–16). A-to-I editing of dsRNAs by ADARs triggers substantial functional changes, in both uninfected and virus-infected cultured cells and intact animals. These changes are caused by editing-induced alterations in RNA sequence and structure. A-to-I editing effectively substitutes a G for an A and hence causes a change in RNA sequence. Such substitutions may affect sequence-dependent processes such as messenger RNA (mRNA) decoding,

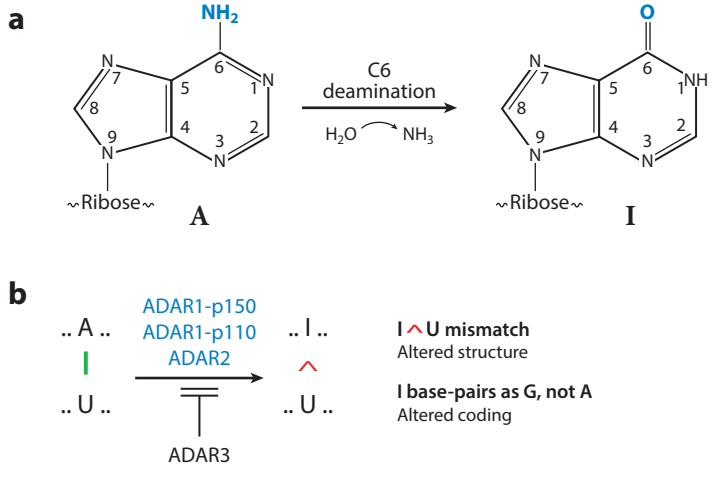


Figure 1

Deamination of A in dsRNA by ADARs. (*a*) C6 deamination of A yields I. (*b*) Adenosine deamination in dsRNA is catalyzed by ADARs. In mammals, there are four ADARs, one interferon-inducible (ADAR1-p150) and three constitutively expressed (ADAR1-p110, ADAR2, and ADAR3). ADAR1-p150, ADAR1-p110, and ADAR2 possess enzymic activity, whereas ADAR3 lacks deaminase activity but can inhibit active ADARs. Abbreviations: A, adenosine; ADARs, adenosine deaminases acting on RNA; dsRNA, double-stranded RNA; G, guanosine; I, inosine. Figure adapted from Reference 15.

precursor mRNA (pre-mRNA) splicing, microRNA silencing, and RNA-dependent replication by viral polymerases. And because an I-U mismatch pair is less stable than an A:U base pair, A-to-I editing also may alter RNA structure-dependent sequence-independent processes such as dsRNA sensing.

There are three *ADAR* genes in mammals (1): *ADAR1* (*ADAR*), *ADAR2* (*ADARB1*), and *ADAR3* (*ADARB2*). These genes encode three enzymically active ADAR proteins: ADAR1-p150 that is IFN inducible, and ADAR1-p110 and ADAR2 that are constitutively expressed. A fourth ADAR protein, ADAR3, does not possess demonstrable deaminase activity. ADAR1 and ADAR2 deaminate both viral and cellular dsRNAs. The biologic effects of the resultant A-to-I substitutions range from antiviral to proviral to none, with the outcome dependent upon the specific virus, the particular ADAR deaminase, and the physiologic conditions (15, 17–19). We consider here the three ADARs, what they are, and what they do, and we describe examples of their effects on virus-host interactions.

ADAR1

There are two isoforms of ADAR1 (**Figure 2**): p150 is IFN inducible and found in both the cytoplasm and nucleus; p110 is constitutively expressed and predominantly if not exclusively found in the nucleus (20). The human *ADAR1* gene maps to chromosome 1q21 (21), and mature transcripts

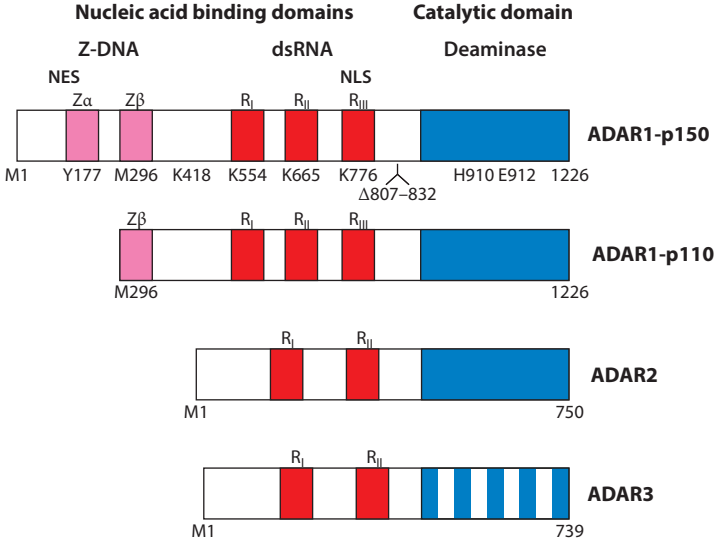


Figure 2

Domain organization of ADAR proteins. The deaminase catalytic domain (*blue*) is present in the C-terminal region of the active ADAR1 and ADAR2 enzymes and the inactive ADAR3 (*blue columns*) protein. Repeated dsRNA-binding domains (*red*) are designated R_I, R_{II}, and R_{III}. The N-terminal region of ADAR1-p150 possesses repeated copies of the Z-DNA-binding domains (*pink*), designated Zα and Zβ. Inducible ADAR1-p150 initiates at M1 and lacks amino acid residues 807–832 due to alternative transcript splicing; constitutive ADAR1-p110 initiates at M296 and includes amino acid residues 807–832. Human ADAR1 is sumoylated at K418; mutation of K554, K665, and K776 impairs dsRNA binding activity of the respective R domain; and mutation of Y177 of Zα impairs DNA binding activity. H910 and E912 are essential residues of the CHAE deaminase catalytic core. Abbreviations: ADARs, adenosine deaminases acting on RNA; dsRNA, double-stranded RNA; NES, nuclear export signal; NLS, nuclear localization signal. Figure adapted from Reference 7.

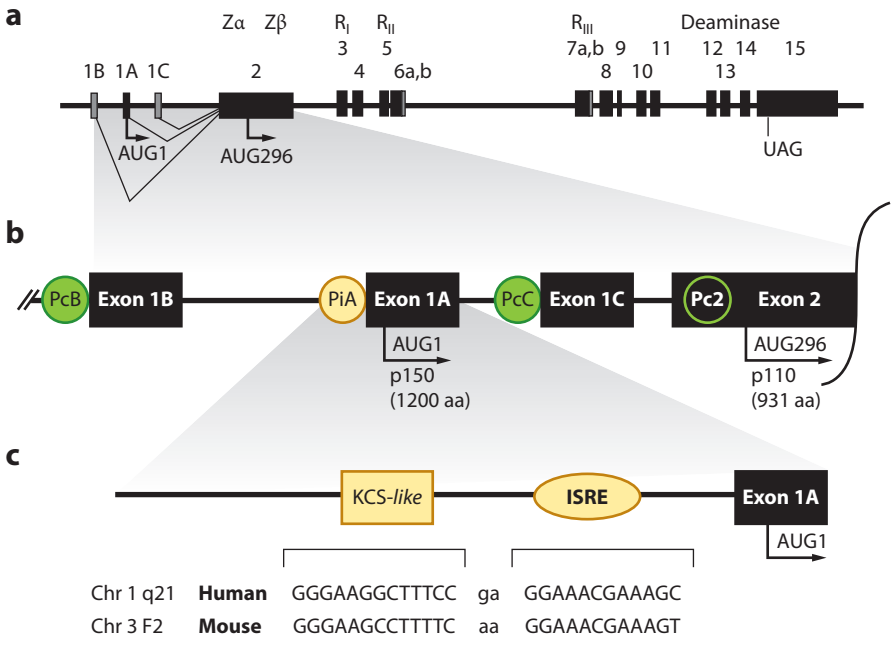


Figure 3

Organization of the human *ADAR1* gene. (*a*) Exon-intron organization of the ~40-kbp human *ADAR1* gene found on chromosome 1q21. Exons 1–15 are indicated by black boxes and include alternative exons 1A, 1B, and 1C and alternative exons 6a and 6b and 7a and 7b. Introns and 5′- and 3′-flanking regions are indicated by solid lines. (*b*) Alternative promoters are indicated by circles and include PiA (*yellow*), which is IFN inducible, and PcB, PcC, and Pc2 (*green*), which are constitutively active. The PiA promoter drives expression of alternative exon 1A-, exon 7b-containing transcripts that encode the 1,200-aa ADAR1-p150 protein that initiates from AUG1 present in exon 1A. Alternative exons 1B and 1C do not contain an AUG; constitutively active promoters drive expression of transcripts that initiate translation of the 931-aa ADAR1-p110 protein from the in-frame AUG296 codon present in exon 2. (*c*) The human *ADAR1* and mouse *Adar1* genes possess highly conserved PiA promoter elements (*yellow*) that drive the IFN-inducible expression of exon 1A-containing transcripts: a 12-bp ISRE found in type I IFN-inducible genes and an adjacent 13-bp KCS-*like* element, which is also found in the protein kinase R promoter. Abbreviations: ADARs, adenosine deaminases acting on RNA; IFN, interferon; ISRE, interferon-stimulated response element; KCS, kinase-conserved sequence. Figure adapted from Reference 17.

include 15 exons (**Figure 3**). The ADAR1 consensus complementary DNA (cDNA) sequence from human cells possesses an open reading frame (ORF) of 1,226 aa (20, 22, 23).

ADAR1 is ubiquitously expressed and inducible by microbial infections (5, 6, 15, 24–27). Expression is driven by alternative promoters (**Figure 3**), one IFN inducible (PiA) and the others constitutively active (PcB, PcC, and Pc2) (28–30). Alternative splicing of the IFN-inducible transcripts produces mRNA beginning with alternative 5′-exon 1A that possesses AUG1, the Met initiation codon for p150 synthesis. Exon 1A-containing ADAR1 transcripts typically have alternative exon 7b and encode a 1,200-aa p150 protein. Constitutively expressed mRNA begins with alternative 5′-exon 1B (or 1C), which lacks an AUG and typically has exon 7a. These transcripts encode a 931-aa p110, whose translation is initiated at AUG296 in exon 2 (28–30). Exon 1A-containing mRNA is optimized to express both p150 and p110 by a leaky scanning translation initiation mechanism (26). However, the majority of p110 is synthesized from exon 1B- or 1C-containing mRNAs. The promoter and exon organization of the mouse *Adar1* gene (24, 27, 31,

32) is conceptually similar to that of the human *ADAR1* gene; mouse p150 is 1,152 aa, and mouse p110 is 903 aa.

Induction of ADAR1-p150 by IFN occurs through Janus kinase-signal transducers and activators of transcription signaling (33) by the transcriptional activator complex STAT1-STAT2-IRF9. The inducible PiA promoter for the human (28) and mouse (34) genes possesses a consensus interferon-stimulated response element (**Figure 3**). The STAT2-dependent, IRF9-dependent induction of *Adar1* by IFN differs between mouse and human cells in the stringency of the requirement for STAT1 (34, 35). The PiA promoter also possesses a kinase-conserved sequence–like element (**Figure 3**) initially identified in the protein kinase R (PKR) promoter that enhances transcriptional activity through Sp factor binding (17).

The p150 and p110 ADAR1 proteins each have three copies of the dsRNA-binding domain (R) in their central region and the deaminase catalytic domain in the carboxy (C)-terminal region (7, 8, 20, 22, 23, 36) (**Figure 2**). R domains bind right-handed, A-form dsRNA in a sequence-independent manner. ADAR1-p150 is an amino (N)-terminally extended version of ADAR1-p110; the additional 295-aa sequence of human ADAR1-p150 includes the Z-DNA/Z-RNA binding (Z)-domain Zα that binds left-handed, Z-form dsDNA and dsRNA. Recognition of CpG repeats and also non-CpG repeats in a broad range of RNA sequences by Zα is accompanied by destabilization of neighboring A-form regions (37). ADAR1 domains for dsRNA-binding and deaminase catalytic activity are functionally distinct (38). Mutation of lysines K554, K665, and K776 present in the ADAR1 dsRNA-binding domains impairs binding of dsRNA (**Figure 2**). Mutation of histidine H910 and glutamic acid E912 in the catalytic domain CHAE sequence of ADAR1 destroys deaminase activity. Sumoylation at K418 reduces ADAR1 deaminase activity (39). Protein kinase B–dependent phosphorylation of ADAR1-p110 at threonine T738, and ADAR2 at T553, reduces deaminase activity; the phosphomimic mutants T738D and T553D show reduced editing activity (40). Stress-activated phosphorylation of ADAR1-p110 by mitogen-activated protein kinases promotes Staufen1-mediated mRNA decay (41). Finally, Y177 is an essential tyrosine residue for function of the Zα domain of p150 (36, 42).

A-to-I editing sites have been identified by multiple strategies, including RNA sequencing. The vast majority of sites occur in repetitive noncoding sequences exemplified by *Alu* sequences in the human transcriptome; ADAR1 is largely responsible for most of these editing events, including the hyperediting of viral and cellular RNAs (6, 8, 25, 43). Editing analysis of total RNA from ADAR1 null cells reconstituted with either p150 or p110 revealed that more than half of the editing sites are edited by p150, with the other half edited by either p150 or p110 (26). ADAR1 and ADAR2 display different substrate selectivity, determined in part by their deaminase domains (44). However, analysis of recombinant chimeric proteins between ADAR1 and ADAR2 and between ADAR1 and PKR illustrates that substrate selectivity is determined by both the RNA binding domains (45) and the deaminase domain (44).

In mice, genetic ablation of *Adar1* gene function by disrupting expression of both p150 and p110 or by disrupting p150 expression alone, or by knock-in of the catalytically dead E861A mutant (corresponding to E912 in human ADAR1), results in embryonic lethality (31, 32, 46–48). This phenotype is largely rescued by a second concomitant knock-out of either the *Mda5* (*Ifih1*) sensor or the *Mavs* adapter (48–50), although the phenotypes of E861A knock-in *Adar1*, *Ifih1* deletion and the *Adar1*, *Mavs* double deletion are not equivalent (48–51).

ADAR2

ADAR2 expression is driven by multiple constitutive promoters and involves alternative splicing of transcripts. Major ADAR2 proteins in human cells predicted from cDNA sequence are 701

and 750 aa (17, 52). ADAR2 possesses two copies of the RNA-binding domain in the N-terminal region; the deaminase catalytic domain is present in the C-terminal region (**Figure 2**). ADAR2 does not have a Z domain. Unlike ADAR1, ADAR2 is not regulated by IFN (6, 7, 19, 53). ADAR2 is phosphorylated by protein kinase C at two sites between the dsRNA-binding domains, S211 and S216. Phosphorylation at these sites affects editing of microRNA (miR)-200 (54). ADAR2 is largely responsible for the highly site-selective A-to-I editing observed in mammalian cells (6, 25).

In mice, genetic ablation of *Adar2* does not result in embryonic lethality. However, *Adar2*-null mice have diminished survival. Postnatal death of mice lacking ADAR2 protein is largely rescued by the knock-in of the already-edited glutamate receptor (GluRB) Q/R-site (55). ADAR2 is required for normal physiology more broadly in mice (56). ADAR2 autoregulates its expression at the level of pre-mRNA splicing. Introns of the U2 type are flanked by 5′-GU–AG-3′ consensus splice-site sequences. Alternative 5′-AU or AA-3′ splice sites can be converted to the canonical splice-site sequence by A-to-I editing, as I is recognized as G by the spliceosome machinery. Such site-specific editing was demonstrated with ADAR2 pre-mRNA, where ADAR2 autoediting converts the AA-3′ sequence to AI-3′ (57). This type of editing affecting pre-mRNA splicing is rare, thus far observed for only a relatively limited number of cellular transcripts (58).

ADAR3

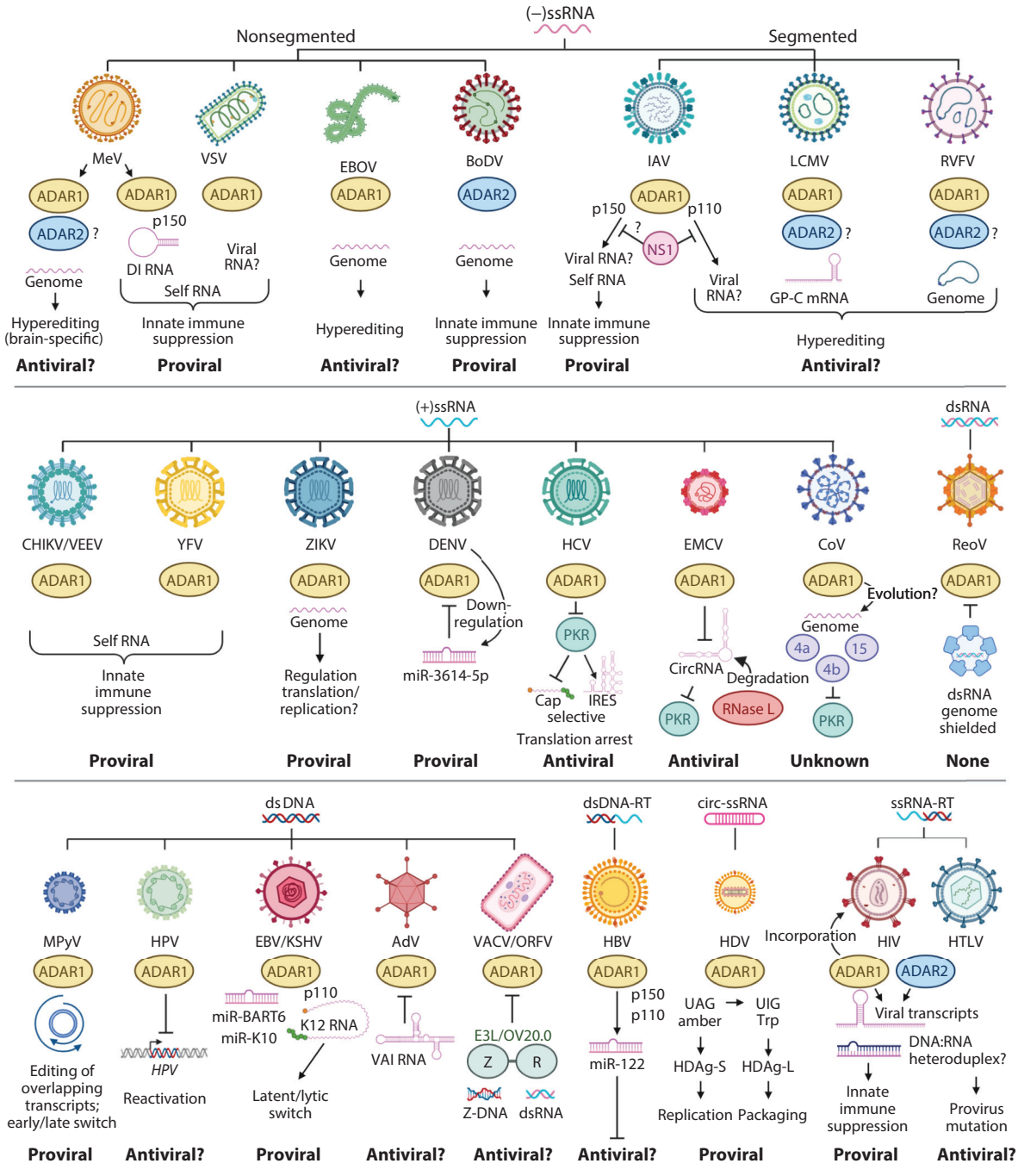
Expression of ADAR3 is restricted to the nervous system; expression is high in the hippocampus and amygdala (59, 60). Human ADAR3 protein, predicted by cDNA sequence, is 739 aa and includes two copies of the R domain in the N-terminal region and a deaminase-like C-terminal domain (17, 59). While wild-type (WT) ADAR3 does not display detectable deaminase catalytic activity (4, 6, 61), mutation of human ADAR3 at five positions (A389V, V485I, E527Q, Q549R, and Q733D) generates an active enzyme (61). Binding of WT ADAR3 to dsRNA structures can affect the editing efficiency of ADAR2, illustrated by the ADAR3 inhibition of GluRB pre-mRNA editing in glioblastoma cells (62). In mice, genetic ablation of *Adar3* does not result in embryonic lethality. However, mice lacking Adar3 display deficits in hippocampus-dependent short- and long-term memory formation (60).

EFFECTS OF ADARs AND A-TO-I DEAMINATION EDITING ON VIRUS-HOST INTERACTIONS

We now consider examples of virus infections affected by ADARs in either an antiviral or proviral manner as summarized by **Figure 4**.

Measles Virus

Among negative-strand single-stranded (ss) RNA viruses (NSVs), much is known about A-to-I editing of measles virus (MeV) RNAs by ADARs (63). The viral genome, like that of other *Mononegavirales*, is transcribed and replicated by a virion-associated RNA polymerase. The MeV *P/V/C* gene-encoded accessory proteins C and V modulate innate immune responses, including synthesis of defective-interfering (DI) RNAs with ds structure that are PKR activators and ADAR substrates. Clustered A-to-I editing (hyperediting) of MeV RNA isolated from persistently infected brain tissue of subacute sclerosing panencephalitis (SSPE) patients (63) was discovered before ADAR enzymes were identified (2, 3). Editing disrupted viral protein production required for particle assembly, suggesting that editing may be linked directly to development of the often-fatal persistent viral infection. This process could be interpreted as the antiviral effect of ADAR. Because MeV exclusively replicates in the cytoplasm, editing likely is performed by cytoplasmic ADAR1-p150.



(*Caption appears on following page*)

Figure 4 (*Figure appears on preceding page*)

Effect of ADARs on virus replication and host responses to viral infection. ADARs are antiviral, proviral, or have no effect on virus infection, depending on the virus-host combination. Representative animal viruses are grouped by the biochemical nature of their genomes. The protein (ADAR1, *yellow*; ADAR2, *blue*) implicated in mediating the response, the viral or host RNA targeted by the ADAR protein, and the mechanism by which viral replication is affected are indicated. Abbreviations: ADARs, adenosine deaminases acting on RNA; AdV, adenovirus; BoDV, Borna disease virus; CHIKV, chikungunya virus; circ, circular; CoV, coronavirus; DENV, Dengue virus; DI, defective-interfering; ds, double-stranded; EBOV, Ebola virus; EBV, Epstein-Barr virus; EMCV, encephalomyocarditis virus; GP-C, glycoprotein precursor; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis delta virus; HIV, human immunodeficiency virus; HPV, human papillomavirus; HTLV, human T cell leukemia virus; IAV, influenza A virus; IRES, internal ribosome entry site; KSHV, Kaposi sarcoma–associated herpesvirus; LCMV, lymphocytic choriomeningitis virus; MeV, measles virus; MPyV, murine polyomavirus; mRNA, messenger RNA; ORFV, Orf virus; PKR, protein kinase R; ReoV, reovirus; RT, reverse transcriptase; RVFV, Rift Valley fever virus; ss, single-stranded; VACV, vaccinia virus; VAI, adenovirus virus-associated RNA-I; VEEV, Venezuelan equine encephalitis virus; VSV, vesicular stomatitis virus; YFV, yellow fever virus; ZIKV, Zika virus. Figure adapted from images created with BioRender.com.

Different from the rare SSPE disease, ADAR1 is generally proviral for acute MeV infection by counteracting PKR activation (64), stress granule formation (65), apoptosis (64), and type I IFN production (66). ADAR1-p150 hyperedits dsRNA-forming DI RNA of C protein–deficient MeV (43, 67, 68), which activates innate immune responses (69, 70). By altering DI RNA secondary structures, ADAR1 interferes with their recognition by dsRNA sensors including PKR (68). Likewise, ADAR1-p150 edits mRNA with *Alu*-duplex structures and prevents autoimmune reactions against self dsRNA (43, 53, 71, 72), suggesting that both viral and self RNA can trigger activation of PKR, MDA5, and oligoadenylate synthetase (OAS) (**Figure 5**), when the total amount of dsRNA exceeds the level that can be efficiently edited by ADAR1-p150 (43, 64–66, 68, 73–76). Notably, ADAR1 also appears to affect some adaptive T cell immune responses by a similar mechanism (77).

Vesicular Stomatitis Virus

Similar to MeV, ADAR1 displays a proviral activity with vesicular stomatitis virus (VSV) (74). While ADAR1 deficiency does not affect virus yield per se (47, 74, 78), ADAR1-deficient cells display stronger PKR activation following treatment with type I IFN and thus mount stronger antiviral responses against VSV (74). IFN treatment may lead to expression of mRNA species with intrinsic dsRNA structures (43, 71, 72) that then activate PKR in the absence of ADAR1 (43, 53, 65, 66, 71). Alternatively, ADAR1 may directly block PKR activation through interactions of their R domains, as seen for some RNA-binding proteins (79, 80). Consistent with this observation, overexpression of ADAR1 enhances VSV replication in cells from WT mice but not from PKR-null animals (73).

Ebola Virus

ADAR-like hypermutations are reported to accumulate in Ebola virus (EBOV) genomes in the glycoprotein gene when passaged in bat cells with high expression levels of ADAR1. However, such hypermutations are not observed following passaging of EBOV in human embryonic kidney (HEK)-293T cells with lower ADAR1 expression (81). Whether the effect is ADAR1 specific or also involves ADAR2 remains unclear. The observation is reminiscent of the hyperediting phenotype seen with MeV (63) and lymphocytic choriomeningitis virus (LCMV) (82) and suggests that ADAR1 has antiviral properties against EBOV and conceivably contributes to control of viral replication in bats.

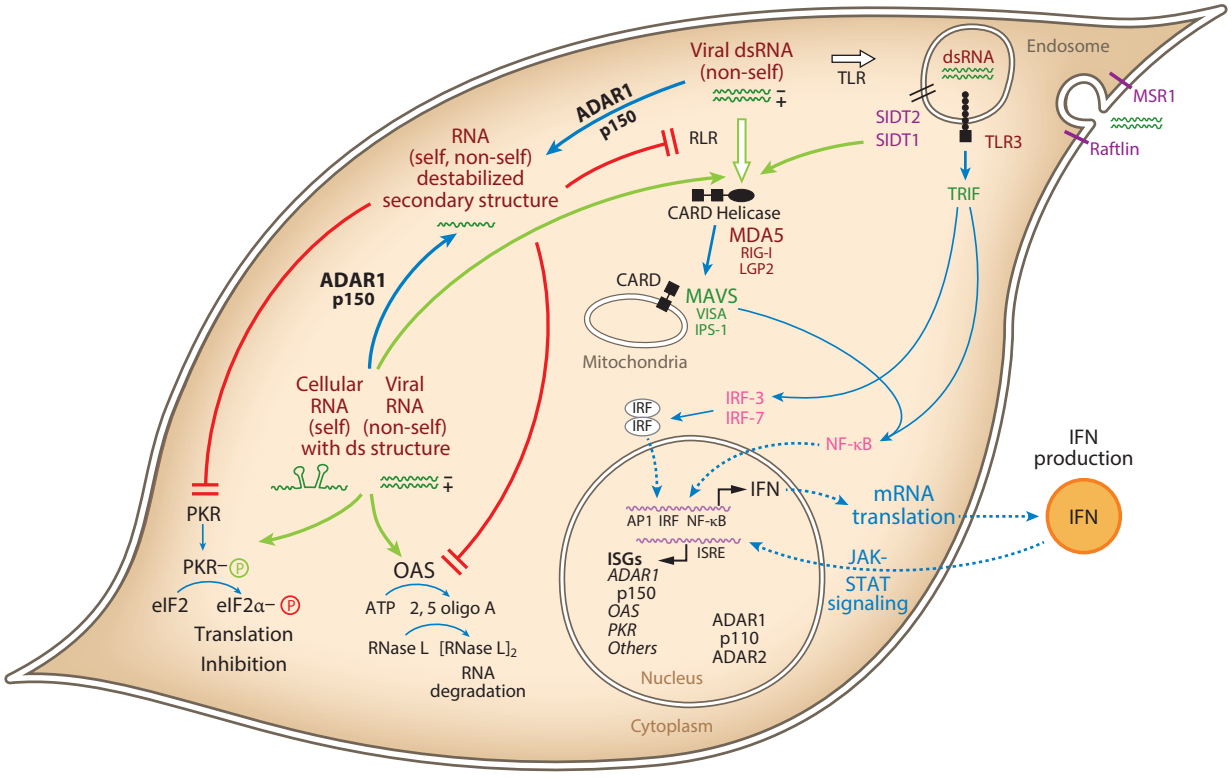


Figure 5

Model for ADAR1 as a suppressor of innate immune responses activated by dsRNA. Cytoplasmic RLRs MDA5 and RIG-I and endosome-associated TLR3 sense intracellular dsRNA to signal via MAVS and TRIF adapters the activation of IRF and NF-κB transcription factors that transcriptionally activate IFN expression. Extracellular viral dsRNAs may be transferred into uninfected bystander cells by the surface receptors Raftlin and MSR1 and internalized and transported to the cytoplasm by SIDT1/2 to trigger innate immune dsRNA sensors. Among the ISGs induced by IFN and JAK-STAT signaling are PKR and OAS, which, when activated by cytoplasmic dsRNA, lead to translation inhibition and RNA degradation. Under conditions of ADAR1-p150 deficiency, cytoplasmic viral (nonself) and cellular (self) RNAs with ds structure accumulate to a concentration surpassing the threshold required for activation of innate immune sensors including MDA5, PKR, and OAS, thereby leading to IFN production and action. In IFN-treated cells, cytoplasmic p150 is elevated, which leads to A-to-I editing and inactivation of cellular (self) dsRNAs that then fall below the threshold concentration necessary for innate immune sensor activation. Viral infection, however, may lead to sufficiently high concentrations of intracellular viral (nonself) dsRNAs that surpass the threshold for dsRNA sensor activation and trigger innate immune responses. Abbreviations: ADARs, adenosine deaminases acting on RNA; ds, double-stranded; IFN, interferon; IRF, interferon regulatory factor; ISRE, interferon-stimulated response element; JAK-STAT, Janus kinase-signal transducers and activators of transcription; mRNA, messenger RNA; OAS, oligoadenylate synthetase; PKR, protein kinase R; RLR, RIG-like receptor; SIDT, systemic RNA interference defective transmembrane; TLR, Toll-like receptor. Figure adapted from Reference 7.

Borna Disease Virus

Borna disease virus (BoDV) is one of the few nonsegmented NSVs that replicates in the nucleus. While both ADAR1 and ADAR2 deficiencies reduce acute BoDV infection, establishment of persistent BoDV infection is reduced only in ADAR2-deficient cells (83). ADAR2-deficient cells exhibit an antiviral state characterized by upregulation of immune and inflammatory response genes, suggesting that ADAR2, like ADAR1, regulates autoimmune responses (43, 53, 71, 72). BoDV nuclear replication is reduced in ADAR2-deficient compared to ADAR2-sufficient cells

(83). In addition, ADAR2 binds BoDV genomic RNA and introduces mutations, albeit at relatively low frequencies (83).

Influenza A Virus

ADAR-editing has multifaceted effects on influenza A virus (IAV) infections. Some IAV strains induce upregulation of ADAR1 but not ADAR2, leading to increased A-to-I editing of cellular (84) and viral (85) RNAs. Moreover, ADAR-characteristic hypermutations are found in genomes of IAV vaccine preparations (86), However, ADAR1-p150 blocks sustained RIG-like receptor signaling (**Figure 5**); p150 is required for efficient IAV replication (87), in line with the reported proviral activity of ADAR1-p150 as a regulator of dsRNA-mediated innate immune responses (43, 64, 74). In contrast, reduced expression of ADAR1-p110 alone leads to an increase in viral replication, indicating a potential antiviral role of the p110 isoform (87). Thus, ADAR1-p110, which is nuclear, seemingly edits viral RNAs leading to an antiviral effect (84, 85), while cytoplasmic ADAR1-p150 limits dsRNA-mediated immune responses. Replicating IAV generates Z-RNAs that subsequently induce necroptosis via Z-DNA-binding protein 1, which possesses a Zα-domain like p150 (88). The IAV nonstructural protein 1 (NS1), a dsRNA-binding protein that is a potent PKR antagonist (15, 89), interacts with nuclear ADAR1 in a dsRNA-binding-independent manner through interactions with the R_I and R_{II} domains (90), suggesting that NS1 functions in reducing antiviral activities of p110 and thereby optimizes IAV replication.

Arenaviruses and Bunyaviruses

ADAR-characteristic A-to-G and U-to-C hyperediting patterns are observed with LCMV of the *Arenaviridae* family and Rift Valley fever virus (RVFV) of the *Phenuiviridae* family. LCMV RNAs are synthesized by an ambisense replication strategy and can form an immunostimulatory dsRNA intermediate (91). ADAR-characteristic hypermutations are observed in the LCMV glycoprotein gene (82) and the RVFV L gene of a strain lacking functional IFN antagonist NSs (92). In both cases, editing is likely antiviral, as it impairs function of edited gene products. Notably, ADAR1-p150 expression is upregulated during LCMV infection (82, 91), and A-to-I-editing of RVFV RNA depends on a functional IFN system (92), which together suggest that the IFN-inducible ADAR1-p150 is responsible for the observed editing.

Alphaviruses

A proviral activity of ADAR1 was identified for two alphaviruses, chikungunya virus (CHIKV) and Venezuelan equine encephalitis virus (VEEV). Replication of both viruses is enhanced in cells overexpressing ADAR1 (93). Curiously, this effect is observed in *Stat1*$^{-/-}$ cells, initially suggesting that it was possibly independent of canonical type I IFN signaling (93). However, some interferon-stimulated genes (ISGs) including *ADAR1* p150 expression do not display a strict requirement for STAT1 (35). CHIKV replication is inhibited by the PKR-mediated stress responses (94). Whether ADAR1 protects CHIKV and VEEV from these responses that otherwise would induce shutdown of viral protein synthesis remains unknown.

Flaviviruses

Flaviviruses, like alphaviruses, are zoonotic arthropod-borne viruses. Yellow fever virus (YFV) is a hepatotropic flavivirus. ADAR1 overexpression has a slight proviral effect on YFV replication (93), similar to CHIKV and VEEV. Zika virus (ZIKV) is a neurotropic flavivirus. Genomic analyses of multiple Old-World strains indicate an overrepresentation of G over A nucleotides at

twofold-degenerated sites and synonymous codons (95, 96). These mutations coincide with regions forming dsRNA secondary structures and are consistent with A-to-I editing (95). ZIKV infections induce ADAR1 expression in neural stem cells and alter their proliferative capacity and neuronal differentiation (97), but it is unclear whether ADAR1 is directly responsible. In ADAR1-deficient cells, ZIKV replication is decreased and PKR activation increased (98). ADAR1-p110 expression rescues this phenotype (98). ZIKV-mediated induction of ADAR1 in neuronal cells may allow strong and neurotoxic viral replication and promote autoimmune responses such as Guillain-Barré syndrome (99). Dengue virus (DENV) induces ADAR1-p150 expression in monocyte-derived macrophages, resulting in downregulation of the antiviral miR-3614-5p (100). Notably, miR-3614-5p overexpression leads to reduction of DENV titers only in ADAR1-sufficient cells, not in ADAR1-deficient cells, indicating that miR-3614-5p function depends on ADAR1 (100).

Hepatitis C Virus

Hepatitis C virus (HCV) is a blood-borne pathogen that replicates in the cytoplasm of hepatocytes, where it hijacks lipid droplets and reorganizes membrane structures for efficient viral replication (101) and innate immune evasion. Pegylated IFN-α therapy increases expression of ISGs including *ADAR1* (102). Increased A-to-I editing of ADAR1 targets, like the phosphodiesterase 8A (PDE8A) transcript (103), counteracts PKR activation (53, 64, 65), which for several viruses has a proviral effect (43, 66, 71, 74, 87). However, for HCV, increased PKR activity is beneficial for viral replication, as PKR-mediated translational arrest selectively blocks 5′-cap-dependent translation, while internal ribosome entry site–dependent translation initiation of HCV RNA is not affected (104). ADAR1 knock-down increases type I IFN responses, moderately stimulates HCV replication in Huh7 cells (105, 106), and leads to reduced sensitivity of HCV replication to IFN (106). In contrast, replication of an HCV replicon in ADAR1-deficient Huh7.5 cells, which express a nonfunctional RIG-I dsRNA sensor, is not altered relative to replication in WT cells (107). Taken together, ADAR1 appears to have antiviral activity against HCV that is achieved by limiting PKR-mediated translational arrest.

Picornaviruses

Encephalomyocarditis virus (EMCV) is a member of the *Picornaviridae* family. Following infection with EMCV, viral circular RNAs (circRNAs) form that are 16–26-bp imperfect RNA duplexes; these circRNAs antagonize PKR activation. circRNAs are degraded by RNase L, a process required for PKR activation during early cellular innate immune responses (108). circRNAs are substrates for ADARs; knock-down of ADAR1 significantly upregulates circRNA expression (109).

Coronaviruses

The *Coronaviridae* family includes the highly pathogenic Middle East respiratory syndrome (MERS) coronavirus (CoV); severe acute respiratory syndrome (SARS) CoV; and SARS-CoV-2, the causative agent of the pandemic outbreak of 2019–2020, causing coronavirus disease 2019 (COVID-19). CoVs produce dsRNA replication intermediates that can activate PKR and other dsRNA-mediated immune responses (110). Innate immune evasion occurs through CoV nonstructural proteins NSP4a, a dsRNA-binding protein (111); MERS-NSP4b and mouse hepatitis virus (MHV)-NSP2, which are phosphodiesterases that antagonize RNase L activation (112, 113); and NSP15, an endoribonuclease required to evade MDA5, PKR, and OAS dsRNA sensors (114). ADAR deficiency leads to a dsRNA-activated OAS/RNase L response that is antagonized by the MHV-NSP2 (113). In genomes of SARS-CoV-2 isolates, nucleotide transitions characteristic of

both ADAR and apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) activity are overrepresented (115, 116), but they do not display the hyperediting phenotype often seen with ADAR activity. Direct evidence for ADAR editing, such as comparative sequence analyses of SARS-CoV-2 propagated in the presence and absence of ADAR1, has not been reported.

Reovirus

Reoviridae viruses have segmented dsRNA genomes and replicate in the cytoplasm. The synthesis of progeny genome dsRNA occurs within a core-like subvirion particle during virion morphogenesis (117), which efficiently shields viral dsRNA from some immune sensors including ADARs. No significant differences are observed in single-cycle reovirus (ReoV) yields in WT and ADAR1-p150-null mouse embryo fibroblasts (MEFs) (47), and *Adar1*$^{-/-}$ cells lacking both p110 and p150 and *Adar2*$^{-/-}$ MEFs (78). While infection of neonatal mice with the neurotropic ReoV Dearing strain results in significant ADAR1 induction in the brain, the induction results in few A-to-I(G) substitutions in virus-infected tissues as determined by high-throughput sequencing (27). Although ReoV expresses a dsRNA-binding protein (σ3), unlike vaccinia virus (VACV) E3L, σ3 has no inhibitory activity against ADAR1 editing of synthetic or natural dsRNA substrates (118). Taken together, these results suggest that ADARs surprisingly do not have a significant effect on ReoV infections, despite the large amounts of viral dsRNA present in reovirus-infected cells.

Polyomavirus

Mouse polyomavirus (MPyV) has an ~5-kb circular dsDNA genome. DNA replication and subsequent virion assembly occur in the nucleus. Temporal regulation of bidirectional gene transcription by cellular RNA polymerase II occurs, giving rise to early and late viral transcripts made from opposite strands of the DNA genome. Late in the replication cycle, early mRNA is downregulated by late-strand RNA facilitating the switch from the production of T antigens to capsid proteins. An overlap between the 3′-ends of early and late transcripts forms a sense-antisense duplex, a viral dsRNA, which undergoes extensive A-to-I(G) substitutions as established by sequence analysis (119, 120).

Knock-down of Adar1 in 3T3 cells leads to a defect in the early-to-late switch and reduced expression of late transcripts (120). However, single-cycle yields and growth kinetics of MPyV are comparable in *Adar1*$^{-/-}$ and *Adar2*$^{-/-}$ cells. Although the expression of early proteins is greater in *Adar1*$^{-/-}$ cells than in *Adar2*$^{-/-}$ cells, the expression of late proteins is comparable in both mutant cell types and parallels comparable viral yields. However, the virus-induced cytopathic effect is greatly enhanced in *Adar1*$^{-/-}$ cells relative to *Adar2*$^{-/-}$ cells. Complementation with Adar1-p110 rescues cells from MPyV-induced cytotoxicity (78).

Human Papillomavirus

Human papillomavirus (HPV) has an ~8-kb circular dsDNA genome that contains six early genes (E1, E2, E4, E5, E6, and E7) and two late genes (L1 and L2) transcribed by host RNA polymerase II in a temporally regulated manner. But unlike MPyV, HPV mRNAs are transcribed unidirectionally from one DNA strand. Ubiquitination of RIG-I mediated by the ubiquitin ligase tripartite motif-containing 25 (TRIM25) promotes dsRNA signaling to produce type I IFN; the HPV16 E6 oncoprotein targets TRIM25 to impair this signaling (121). ADAR1 regulates the expression of HPV proteins in keratinocyte cells (SiHa) in which the HPV16 genome is integrated (122, 123). ADAR1 knock-down upregulates HPV mRNA and protein (E1 and E7) expression. No A-to-I editing, however, is observed in HPV transcripts. It is postulated that downregulation of

ADAR1 overcomes innate immune check-point blockade, thus facilitating enhanced viral expression (122). Furthermore, a genetic association between ADAR1 and HPV-induced dysplasia recurrence in HPV/human immunodeficiency virus (HIV) coinfected individuals has been observed using ADAR1 haplotype analysis, although the contribution of HIV infection was not excluded (122).

Human Herpesviruses

Herpesviridae are large, enveloped viruses with linear dsDNA genomes ~125–250 kb in size. Viral DNA replication occurs in the nucleus, as does transcription by cellular RNA polymerase II. Epstein-Barr virus (EBV; human herpes virus 4) and Kaposi sarcoma–associated herpesvirus (KSHV; human herpes virus 8) transcripts include several viral long noncoding RNAs (vlncRNAs) and miRs. These RNAs modulate virus-host interactions and maintain viral latency or mediate the switch to the lytic cycle. Site-specific A-to-I(G) editing occurs of RNA transcripts encoded by these two human herpesviruses (124–126). Extensive A-to-I editing also is seen in malacoherpesvirus-infected mollusks, possibly by an ADAR1 homolog (127).

Epstein-Barr Virus

Of the several EBV miR genes now known, primary transcripts of four miRs undergo site-specific A-to-I(G) editing: pri-miR-BART6, pri-miR-BART8, pri-miR-BART16, and pri-miR-BHRF1 (125). The frequency of editing by ADAR is greater with miR-BART6 relative to the others (125). miR-BART6 targets the DICER nuclease, and miR-BART6 binding to the DICER mRNA 3′ untranslated region (UTR) diminishes DICER expression, affecting mature miRs and influencing EBV latency. A-to-I(G) editing of the pri-miR-BART6 along with a deletion of three U residues causes the complete blockade of the Drosha cleavage step, eliminating the corresponding pre- and mature miR. However, editing alone is sufficient to inhibit the loading of miR-BART6-5P onto a functionally active miRNA-induced silencing complex (125). ADAR1 seems responsible for this editing based on enzyme expression levels. EBV-encoded miR-BART3 likewise undergoes A-to-I(G) editing (128), likely by ADAR1. EBV also expresses vlncRNAs during reactivation (126). One of these, oriPtLs, is A-to-I(G) hyperedited by ADAR1, consequently affecting viral lytic gene expression and viral DNA replication (126).

ADARs also edit cellular as well as viral miRs. A-to-I editing by ADAR1 and ADAR2 can affect the processing of primary miR transcripts (pri-miR) or the targeting of mature miRs (129, 130). It is estimated that ~20% of the pri-miRs are edited by ADARs (131). A-to-I editing of human miRs, likely by ADAR2, often is enriched in seed sequences and significantly hypoedited in glioblastoma (132).

Kaposi Sarcoma–Associated Herpesvirus

KSHV causes two acquired immunodeficiency syndrome–associated malignancies, Kaposi sarcoma and primary effusion lymphoma. The viral K12 transcript is abundantly expressed and encodes three Kaposin proteins (A, B, and C). The region containing the ORF for Kaposin A also encodes miR-K10 and has tumorigenic potential (133, 134). Genome position 117990 falls within this region and is an A in the viral DNA but a G in RNA transcripts, indicating ADAR editing. This editing results in the substitution of a serine for a glycine in the Kaposin A protein. The change also affects miR-K10 with the potential to alter mRNA targeting. The biologic consequence of these changes is the elimination of transforming activity associated with the unedited A at 117990 and the restoration of lytic replication associated with a G(I) at this position (124). Purified ADAR1-p110 selectively edits the A at position 117990 of K12 RNA

(124). ADAR1 isoforms increase throughout the viral lytic cycle progression, in parallel with an increase in the relative amount of edited Kaposin transcripts (135). Genetic recoding of mRNA decoding by ribosomes such as that observed with the Kaposin transcript is seen relatively infrequently with either viral or cellular mRNAs. Among the first and best-characterized examples of highly selective editing to change codons are the cellular pre-mRNAs for GluRB and 5HT2CR serotonin receptor pre-mRNAs and hepatitis delta virus (HDV) RNA (136–139).

Adenovirus

Adenoviruses (AdVs) have linear dsDNA genomes ~30–36 kb in size. Viral DNA replication and transcription occur in the nucleus of infected cells. AdV mRNA transcripts are not known to be edited by ADARs. However, the small viral AdV virus-associated RNA-I (VAI) RNA transcribed by cellular RNA polymerase III is a potent inhibitor of ADAR1-mediated A-to-I(G) editing activity (140). Endogenous ADAR1 present in extracts from IFN-treated human U cells, or ADAR1-p150 or p110 ectopically expressed in COS cells, is inhibited by VAI RNA (140). VAI RNA is an antagonist of PKR and eukaryotic initiation factor-2α (eIF2α) phosphorylation; VAI RNA is required for efficient synthesis of both viral and cellular proteins at late time points after infection (141). Analysis of VAI RNA mutants suggests that the central domain of VAI is important in the antagonism of both ADAR1 and PKR but that the interactions of VAI with ADAR1 and PKR, while similar, are not equivalent with mutants that do not inhibit PKR but do inhibit ADAR1. VAI is not a detectable substrate of ADAR1 (140). The inhibitory activity of VAI is proposed to occur by its direct binding to the dsRNA-binding motifs of ADAR1, thus impeding its catalytic activity or otherwise blocking the binding of RNA substrates. It is not known whether VAI RNA inhibits the activity of ADAR2.

Poxviruses

Poxviruses including VACV have linear dsDNA genomes ~200 kb in size. Unique among DNA viruses, replication of poxviruses occurs in the cytoplasm. Even though significant amounts of dsRNA are formed during VACV infection (14), none of the VACV transcripts are known to undergo A-to-I(G) editing. A viral dsRNA-binding protein, E3L, is a potent inhibitor of not only PKR (142, 143) but also ADAR1 (118). E3L has a single Z domain and a single R domain. One possible mechanism by which E3L impairs ADAR1 activity could be the sequestration of the RNA substrate (118). The carboxy-proximal R domain of E3L is essential for ADAR1 inhibitory activity. While deletion of the N-terminal Z domain has no effect on ADAR1 activity, disruption of the Z domain by double substitution of two conserved amino acids abolishes ADAR1 inhibition. Mutations decreasing Z-DNA binding of E3L also correlate with decreased viral pathogenicity. A chimeric VACV incorporating a related protein that does not bind Z-DNA is not pathogenic, but a mutation that allows Z-DNA binding generates a lethal virus. The capacity to bind the Z conformation is thus essential to E3L activity (144).

Orf virus (ORFV) is a poxvirus that infects sheep and goats. ORFV protein OV20.0 is an ortholog of the VACV E3L protein. OV20.0, like E3L, suppresses innate immune responses in part by inactivating PKR (145). ADAR1 plays a proviral role in ORFV infections by antagonizing PKR activation (145).

Hepatitis B Virus

Hepatitis B virus (HBV) has an ~3.2-kb relaxed partially dsDNA genome that is replicated by reverse transcription of an RNA intermediate. Nuclear covalently closed circular viral DNA (cccDNA) produces the replicative intermediate pregenomic RNA (pgRNA) and viral mRNAs.

HBc nucleocapsid protein, polymerase P protein, and pgRNA are assembled into a nucleocapsid, and the P converts pgRNA into relaxed circular DNA by its reverse transcriptase activity. Type I and III IFNs reduce nuclear cccDNA and HBV RNA levels in HBV-infected hepatocytes (146, 147). ADAR1 affects the replication of HBV, although the implicated mechanism differs between studies (147, 148).

ADAR1 inhibits HBV replication in hepatocyte Huh7 cells (147) by enhancing mirR-122 processing and hence increasing miR-122 levels that then downregulate HBV RNA. Both ADAR1-p110 and p150, but not ADAR2, reduce HBV RNA. A-to-I editing activity of ADAR1 is not required; WT ADAR1 and the E912Q mutant both decrease HBV RNA production. Induction of ADAR1 by IFN-α also reduces HBV RNA levels, whereas knock-down or knock-out of ADAR1 increases HBV RNA (147). In contrast to these findings implicating an miR-based antiviral mechanism for ADAR1 against HBV (147), ADAR1 also was reported to stimulate through its deaminase domain HBV DNA replication in hepatocellular carcinoma cells (148). Overexpression of ADAR1 promotes the replication of all four HBV genotypes.

The single nucleotide polymorphism rs4845384 in the 5′ UTR of the ADAR1 gene is associated with HBV clearance (149, 150). The allele rs4845384AA is associated with lower expression of ADAR1 and poorer response to IFN therapy compared with non-AA alleles. ADAR1 mRNA levels also are lower in persons with chronic hepatitis B compared with healthy individuals. Taken together, these findings suggest an antiviral role for ADAR1 in the context of HBV infection (147, 149, 150).

Hepatitis Delta Virus

HDV is a defective human satellite virus requiring coinfection with HBV for replication (151). The genome of HDV is an ~1.7-kb circRNA that forms a rod-like imperfect dsRNA duplex transcribed apparently by a cellular DNA-dependent RNA polymerase by an unknown mechanism using an RNA template. HDV RNA encodes two forms of delta antigen: small delta antigen (HDAg-S) and large delta antigen (HDAg-L). Highly site-selective ADAR1-mediated editing of HDV RNA allows for HDAg-L synthesis: An amber UAG translation stop codon is converted to a UIG tryptophan codon. The A-to-I editing of HDV occurs on antigenome RNA in the nucleus primarily by ADAR1-p110 (152, 153). In the absence of editing, the HDAg-L protein is not synthesized, and HDV genome RNA is not packaged (154). Triggering of innate immune responses by HDV RNA occurs in part via the MDA5 sensor. However, innate immunity seems not to impair HDV while inhibiting HBV (151). Under normal physiologic conditions the amber/W-site editing is proviral, although increased expression of ADAR1-p150 by IFN treatment, or increased expression of either ADAR1 or ADAR2 by ectopic expression, causes an increase in HDV RNA editing and inhibition of HDV replication (155).

Human Immunodeficiency Viruses

Multiple IFN-induced cellular proteins act as restriction factors against HIV type 1 (HIV-1) (89, 156), including the APOBEC3 family of deoxycytidine deaminases, tetherin that restricts virion release, TRIM5α that recognizes the capsid lattice and mediates disassembly, myxovirus B, a dynamin-like GTPase that prevents nuclear accumulation of HIV-1 provirus, and PKR. In contrast to these antiviral ISGs, ADAR1 is a proviral ISG and enhances HIV-1 gene expression and replication (157–159).

Ectopic overexpression of WT ADAR1 but not the catalytic mutant (**Figure 2**) increases expression of HIV-1 capsid CA protein and virion production, whereas ADAR1 knock-down

decreases viral protein expression (157). In cells overexpressing ADAR1, A-to-G substitutions characteristic of ADAR editing are detected in the Tat and Rev encoding sequences in the vicinity of Rev response element and in the 5′ untranslated region but not around the *trans*-activation response element (157, 159). These results suggest that the proviral effect of ADAR1 is dependent at least in part on ADAR1 catalytic activity. However, other studies indicate that ADAR1 enhances HIV-1 replication by both editing-independent and editing-dependent means (157, 159). Overexpression of ADAR1-p150 enhances viral protein expression in a manner independent of deaminase activity but dependent on the dsRNA-binding domains. Although differences are observed between HEK-293T and COS7 cells in the apparent requirement for editing activity for the HIV-1 proviral effects, ADAR1 deficiency in cell lines and primary macrophages leads to decreased HIV-1 gene expression and virus replication and a type I IFN signature (157, 159, 160). ADAR2 also displays HIV-1 proviral activity and enhances HIV-1 replication (161), but whether the ADAR1 and ADAR2 activities toward HIV-1 are mechanistically similar or different is not yet clear.

One mechanism by which ADAR1 enhances the replication of multiple different RNA viruses, including HIV-1, is inhibition of PKR (7, 15, 162). ADAR1 interactions with PKR, and decreased PKR activation and eIF2α phosphorylation, occur under conditions of enhanced HIV-1 protein synthesis (158, 159, 163). ADAR1 also interacts with the HIV-1 p55 group-specific antigen, a structural protein (164). Endogenous ADAR1 present in T cells is incorporated into virions purified from HIV-1-infected T lymphocytes. The function of the incorporated ADAR1 has not yet been elucidated. It is possible that incorporation of ADAR1 into virions (164) restricts HIV, perhaps conceptually similar to the restriction mediated by APOBEC3 encapsidated into HIV virions that subsequently deaminates cytosines to uracils in viral cDNA (165).

Biochemical studies have shown DNA editing of DNA:RNA heteroduplex structures by ADARs at deoxyA-C mismatches in model substrates and by ADAR1 and ADAR2 with mutations (ADAR1 E1008Q and ADAR2 E488Q) in their base flipping region (166). It is unknown whether ADARs can edit 2′-deoxyadenosine in the DNA strand of retrovirus DNA:RNA heteroduplex structures. If this were to occur, ADARs potentially could negatively affect retrovirus replication by introduction of A-to-I(G) mutations into the proviral DNA. Examination of the ADAR1-ribonucleoprotein complex during HIV-1 replication led to the finding that ADAR1 also acts as a suppressor of LINE1 (long interspersed nuclear elements 1, or L1) retrotransposition (167). Although ADAR1 binds the basal L1 ribonucleoprotein complex, editing does not appear responsible for the inhibition of L1 retrotransposition (168).

Other Retroviruses

Sequence changes characteristic of hyperediting by ADARs were reported in the mid-1990s for two retroviruses other than HIV-1: Rous-associated virus and avian leukosis virus (169, 170). The A-to-G mutations were found in the U3 long terminal repeat region and an inverted repeat region where nearly 50% of the adenosines were substituted with guanosines. It remains unknown which of the ADARs is responsible for these A-to-G mutations. Extensive A-to-I(G) hyperediting also is observed with human T cell leukemia virus type 2 (HTLV-2) and simian T cell leukemia virus type 3 and is attributed to ADAR1 (171). ADAR1 enhances HTLV-1 and HTLV-2 infection of T lymphocytes, a proviral effect that is independent of ADAR1 catalytic activity and again thought to occur by PKR antagonism (172).

MODULATORS OF ADARs

Studies of poxvirus E3L and AdV VAI gene products first demonstrated that ADAR editing activity could be inhibited by a heterologous RNA-binding protein or structured RNA. However, the

full significance of the inhibition by E3L and VAI is not yet established, as neither VACV nor AdV transcript editing has been described. Conceivably, the physiologic effects in part are independent of editing activity. Proteins and RNAs from many viruses including the NS1 protein of influenza virus affect PKR activity (15, 80, 89, 173); conceivably, some of them also affect ADAR activity, either RNA binding or enzymic or both. In contrast to VACV E3L that antagonizes ADAR1, the influenza virus NS1 and DENV NS3 proteins interact with ADAR1 in a dsRNA-binding independent manner to enhance editing activity (90). ADAR1 and PKR are recognized by nonoverlapping domains of NS1 (90).

Cellular antagonists of ADAR editing activity likewise are known. For example, binding of ADAR3 to the substrate GluRB pre-mRNA impairs ADAR2 editing of the Q/R site (62). ADAR1 and ADAR2 function as dimers; hence, they are likely subject to dominant-negative effects (174, 175). From a screen for repressors of editing, RNA binding proteins SRSF9 and RPS14 were identified that repress ADAR2 editing (176, 177). Multiple proteins were identified as regulators of ADAR1 editing through the use of RNA-sequencing and global protein-RNA binding data (178). These include TDP-43, Drosha, nuclear factor 45/90, and Ro60 that affect editing using multiple mechanisms including regulation of *ADAR1* expression, interaction with ADAR1 protein, and binding to *Alu* RNA elements. DExH-box helicase 9 binds to inverted repeat *Alu* elements. ADAR1-p150, but not p110, is an RNA-independent interaction partner (179). BioID followed by mass spectrometry identified regulatory proteins of ADAR1 and ADAR2, including four dimerization zinc finger domain–containing proteins (180, 181). ADAR1-p110 competitively inhibits binding of Staufen1, a cellular dsRNA-binding protein, to 3′-UTR dsRNAs and antagonizes Staufen1-mediated mRNA decay (41). Whether cellular antagonists or agonists of ADARs are affected by viral infection has not been reported.

VIRAL DOUBLE-STRANDED RNA IN UNINFECTED BYSTANDER CELLS

dsRNA structures are produced in virus-infected cells by multiple mechanisms depending on the specific virus and its genome expression strategy. For example, ssRNA viruses produce dsRNA as part of the replicative intermediate or DI RNAs during RNA replication (43, 65, 74, 182), dsDNA viruses produce opposing transcripts that overlap in part (119, 120), and both viral and cellular transcripts can include regions with inverted complementary sequence (5, 53). These intracellular viral dsRNAs can activate cytoplasmic innate dsRNA sensors, and in some cases, they are suppressed by ADAR1 (**Figure 5**). Infected cells also can transfer extracellular viral dsRNAs, as exemplified by EMCV and herpes simplex virus type 1infections (183), to uninfected bystander cells by cell-surface receptors together with systemic RNA interference defective transmembrane (SIDT) proteins. SIDT1/2 transport dsRNA from endocytic compartments into the cytoplasm, thereby triggering dsRNA sensors (183, 184). SIDT2 loss results in impaired RIG-receptor signaling and impaired IFN production (183). It is unknown whether such extracellular dsRNA entering into bystander cells also is subject to suppression by ADAR-mediated deamination as with dsRNA synthesized and acting within virus-infected cells.

SUMMARY: CHALLENGES AND OPPORTUNITIES

The year 2020 brought a multitude of challenges and revealed opportunities, for science and for society. It was not one of the best years. There was considerable hardship. Lives were upended by and lost due to the COVID-19 pandemic, social injustices, and economic hardships. But with resilience, focus, and dedication, and by working together and with the power of science, we hope to

soon benefit from the development of effective vaccines and immunization against SARS-CoV-2. For ADARs and A-to-I editing, there too are challenges and opportunities to consider as we move forward. While much has been learned about the biochemical mechanisms by which ADARs mediate biologic change, much remains to be discovered about their functions during viral infections.

For ADAR1, a major and potentially sole essential role of p150 is to suppress auto-triggering of innate immune IFN responses mediated by cytoplasmic dsRNA sensors including MDA5, PKR, and OAS. But whether these suppressive responses are the sole result of creating I-U mismatches that destabilize dsRNA structures, or whether I acts in another manner as a tag to signal responses, remains an open question. The finding that ADAR1 plays such a key role in regulating both auto-triggered innate responses in uninfected cells and responses activated by viral dsRNA in infected cells presents opportunities to control the innate response. Perhaps development of pharmacologic agents that impair ADAR1 provides an opportunity to modulate the robustness of innate immune responses. Advances in sequencing strategies together with the use of model organisms and cell-culture systems possessing deficiencies in ADARs continue to provide insights into the functional importance of ADARs under a variety of conditions, including viral infection and IFN treatment. It will be important to gain further understanding of the roles that individual ADARs play and the extent, if any, to which their activities are redundant. Also, further understanding is needed about how the expression of individual ADARs is regulated, in addition to transcriptional activation of ADAR-p150 by IFN and enzymic activity by post-translational modifications including sumoylation and phosphorylation. The biochemical basis for the substrate selectivity of ADAR1 and ADAR2 enzymes and the functions of the repeated nucleic acid binding R and Z domains are not yet fully delineated. Nor is it fully resolved how, and under what conditions including infection, ADARs are regulated by interactions with cellular and viral dsRNA-binding proteins; by nucleic acids, as illustrated by the viral VAI RNA; and by other modifications of A such as reversible N6 methylation and how this may influence the action of ADARs whose C6 deamination is irreversible. Much has been learned about the roles that ADARs play in biology. But much remains to be learned about their importance, both as nucleic acid binding proteins and as enzymes that functionally substitute a G for an A.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank the many investigators in the ADAR and virology fields for their contributions that made this review possible. We apologize that, because of space constraints, we are not able to cite all studies that have contributed to our understanding of ADARs. Work from the authors' laboratories was supported by the US National Institutes of Health (National Institute of Allergy and Infectious Diseases), the German Research Foundation Collaborative Research Center 1021, and the German Federal Ministry of Health.

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