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MINIREVIEW

Enhancement of Replication of RNA Viruses by ADAR1 via RNA Editing and Inhibition of RNA-Activated Protein Kinase▽

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Adenosine deaminase acting on RNA 1 (ADAR1) is a double-stranded RNA binding protein and RNA-editing enzyme that modifies cellular and viral RNAs, including coding and noncoding RNAs. This interferon (IFN)-induced protein was expected to have an antiviral role, but recent studies have demonstrated that it promotes the replication of many RNA viruses. The data from these experiments show that ADAR1 directly enhances replication of hepatitis delta virus, human immunodeficiency virus type 1, vesicular stomatitis virus, and measles virus. The proviral activity of ADAR1 occurs through two mechanisms: RNA editing and inhibition of RNA-activated protein kinase (PKR). While these pathways have been found independently, the two mechanisms can act in concert to increase viral replication and contribute to viral pathogenesis. This novel type of proviral regulation by an IFN-induced protein, combined with some antiviral effects of hyperediting, sheds new light on the importance of ADAR1 during viral infection and transforms our overall understanding of the innate immune response.

The adenosine deaminases acting on RNA (ADARs) are double-stranded RNA (dsRNA) binding enzymes that catalyze RNA editing of cellular and viral dsRNAs by deamination, which converts adenosines into inosines (6, 22, 54). Inosine is recognized as a guanosine, and thereby deamination alters the sequence- or structure-specific recognition of RNAs, their translation, and, consequently, the amino acid sequences of several proteins. This process also affects noncoding RNA, and the modification of microRNA (miRNA) sequences is very important in the RNA interference (RNAi) pathway that regulates posttranscriptional gene expression (35, 53, 54). In vertebrate cells, there are three genes that code for the ADAR1, ADAR2, and ADAR3 proteins. The mammalian *Adar1* gene encodes two forms of the ADAR1 protein: the interferon (IFN)-inducible ~150-kDa form (p150) found in both the cytoplasm and the nucleus and the constitutively expressed ~110-kDa form (p110) found only in the nucleus (40, 90). These two forms are generated through alternative promoters (one of which is IFN inducible) and alternative splicing of exon I (27). Both forms are active deaminases with a C-terminal catalytic deaminase domain, three centrally located dsRNA binding domains (dsRBDs), and either one (p110) or two (p150) Z-DNA binding domains (Z-DBDs) at the N terminus (33, 34) (Fig. 1). ADAR2 has two dsRBDs and a deaminase domain, which mediates the RNA-editing activity. ADAR3 has a similar structure and is expressed specifically in brain cells, but its deaminase activity has not been demonstrated (54).

Modifications by ADAR enzymes in coding and noncoding dsRNAs have the potential to affect several biological processes (19, 35, 46). ADAR-edited transcripts are produced mainly in the central nervous systems of mammals, *Drosophila*, and squids, where they increase the diversity of proteins produced from a single gene (59, 61, 72). A large, ever-growing number of introns, untranslated regions, and miRNAs have been shown to be edited by ADARs, which suggests a wide role in posttranscriptional processes (35, 53, 54). ADAR1 is essential to embryogenesis, as shown by the early lethality of embryos with a defect in hematopoiesis in the fetal liver and stress-induced apoptosis (82, 83). Furthermore, ADAR1 is an essential regulator of hematopoietic stem cell maintenance and a suppressor of IFN signaling via downregulation of IFN-inducible transcripts. This role likely protects organisms from the excessive IFN activation associated with autoimmune disorders, cancer, and chronic inflammation (30).

RNA-editing patterns characteristic of ADAR enzymes have also been observed in several viral RNAs, including those of hepatitis delta virus (HDV) (10, 45), human immunodeficiency virus type 1 (HIV-1) (17, 62), human respiratory syncytial virus (48), influenza virus (74, 78), Epstein-Barr virus (EBV) (36), Rift Valley fever virus (75), measles virus (MV) (2, 12, 13, 75), lymphocytic choriomeningitis virus (LCMV) (92), polyomavirus (42), and *Drosophila* sigma virus (9). In addition, ADAR-type sequence changes have been observed in hepatitis C virus (HCV) (76), human herpesvirus 8 (HHV8) (23), mammalian parainfluenza virus 3 (50), avian leukosis virus (28), and avian Rous-associated virus type 1 (20), providing indirect evidence that ADARs likely influence the replication of these viruses by RNA editing (Table 1).

As an IFN-induced protein, ADAR1 p150 is expected to be

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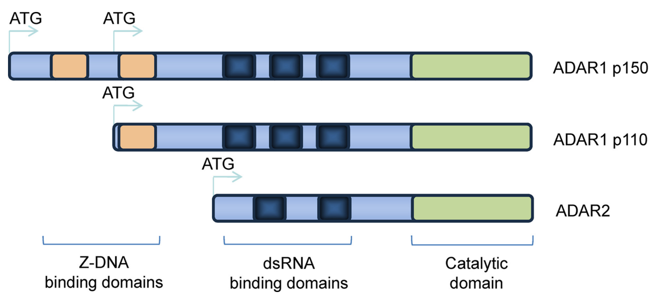

FIG. 1. Schematic representation of ADAR1 p150, ADAR1 p110, and ADAR2. ADAR1 p150 has two Z-DBDs, three dsRNA binding domains, and a catalytic domain. ADAR1 p110 is generated through an alternative promoter and alternative splicing of exon I and is lacking the first Z-DBD. ADAR2 has two dsRNA binding domains and a catalytic domain. Blue arrows indicate the translation initiation sites.

part of the cell response against viral infection and to block viral replication (29, 64, 70). While this antiviral activity seems likely with the HCV replicon, LCMV, and polyomavirus, evidence of wide involvement of ADAR1 as part of the innate immune response against virus infections is lacking. In contrast, ADAR1 interferes with DNA sensors, allowing downregulation of innate immune responses that in excess could be harmful to the host (85). Furthermore, an increasing number of studies have demonstrated that ADAR1 enhances the replication of several RNA viruses (Table 1). Such effects are seen as part of the HDV replication cycle for optimal production of the satellite viral particles (38, 39) and were recently observed with HIV-1 (14, 17, 62), MV (80), and vesicular stomatitis virus (VSV) (43, 52). In the examples described below, experiments clearly demonstrate that ADAR1 is responsible for the enhancement of viral replication via two mechanisms: RNA editing and inhibition of the dsRNA-activated protein kinase (PKR).

ADAR1 ENHANCES THE REPLICATION OF RNA VIRUSES VIA RNA EDITING

RNA editing, possibly due to ADARs, is found in a number of viruses but has been well analyzed for only a few of them. Several lines of evidence clearly show that ADAR1 has direct proviral effects via RNA editing: ADAR1 is required for optimal HDV replication (38, 39), while ADAR1 and ADAR2 promote the production of HIV-1 virions (17, 18, 62).

Hepatitis delta virus. HDV is a subviral satellite of hepatitis B virus (HBV) that increases the severity of HBV-related disease (66). The RNA genome of HDV is a single-stranded (ss) circular molecule that can resemble an imperfect dsRNA with short (~15-bp) double-stranded regions interspersed with numerous mismatches, bulges, and internal loops. The hepatitis delta virus antigen (HDAg) is the sole HDV protein. HDV produces two forms of HDAg (HDAg-S and HDAg-L) that have distinct roles in the viral replication cycle. The HDV replication cycle begins with the production of HDAg-S, which is encoded by the infectious viral genome and supports viral RNA replication (77). The large form is subsequently produced by RNA editing and the specific deamination of adenosine 1012 in the antigenome. The edited adenosine is within the amber stop codon (UAG) that terminates HDAg-S synthesis. After replication and transcription, deamination of this adenosine to inosine leads to the production of an mRNA in which the UAG amber termination codon has been changed to UGG (tryptophan [W]), thereby extending the open reading frame to encode HDAg-L (11, 45). Because of the codon

TABLE 1. Viruses affected or likely affected by ADAR activity

Virus	Type	Host(s)	ADAR activity[a]	ADAR effect[b]	Reference(s)
Avian leukosis virus	RNA	Birds	RNA editing, possibly due to ADAR	ND	28
Epstein-Barr virus	DNA	Humans	RNA editing	Proviral via editing of viral miRNAs	36
Hepatitis C virus (replicon)	RNA	Humans	RNA editing, possibly due to ADAR	Antiviral	76
Hepatitis delta virus	Satellite RNA	Humans	RNA editing	Optimal[c]	10, 45
Human herpesvirus 8	DNA	Humans	RNA editing	Proviral via editing of viral miRNAs	23
Human immunodeficiency virus type 1	RNA	Humans	RNA editing; PKR inhibition	Proviral	14, 17, 18, 62
Human respiratory syncytial virus	RNA	Humans	RNA editing, possibly due to ADAR	Proviral through antibody escape	48, 67
Influenza virus	RNA	Mammals, birds	RNA editing; NS1-ADAR1 binding	ND	51, 74, 78
Lymphocytic choriomeningitis virus	RNA	Mammals	RNA editing, possibly due to ADAR	Possibly antiviral via loss of protein function in heterologous system	92
Measles virus	RNA	Humans	PKR inhibition; RNA editing	Proviral; possibly antiviral via hyperediting and persistence in brain	12, 13, 60, 75, 80
Parainfluenza virus 3	RNA	Mammals	RNA editing, possibly due to ADAR	ND	50
Polyomavirus	DNA	Mammals	Antisense RNA editing, possibly due to ADAR	Possibly antiviral via RNA nuclear retention	42
Rift Valley fever virus	RNA	Mammals	RNA editing	ND	75
Rous-associated virus type 1	RNA	Birds	RNA editing, possibly due to ADAR	None on replication; increased transforming activity	20
Sigma virus	RNA	*Drosophila*	RNA editing, possibly due to ADAR	None detected	9
Vesicular stomatitis virus	RNA	Mammals	PKR inhibition	Proviral	43, 52

[a] NS1, nonstructural protein 1.
[b] ND, not determined.
[c] Both antiviral and proviral effects of ADAR result in optimal replication.

change produced by this editing event, the editing site is called the amber/W site.

HDV-editing activity is accomplished by ADAR1 p110 in the nuclei of HuH-7 hepatoma cells, and the secondary structure of HDV RNA determines the level of editing (10, 44, 63, 88). Experiments using knockdown or overexpression of ADAR1 were conducted to determine to what extent the editing activity is essential for viral RNA replication. Short interfering RNA (siRNA)-mediated knockdown of ADAR1 expression led to decreased HDV amber/W editing and HDAg-L expression, as well as to reduced virus production (39). When ADAR1 or ADAR2 was overexpressed, the amber/W site hyperediting induced excessive production of HDAg-L and extensive non-amber/W site editing with a concomitant production of dominant negative HDAg variants (38). In addition, IFN treatment of HuH-7 cells induced the production of ADAR1 p150, which was able to edit the amber/W site and further increase HDAg-L production (31). These changes were detrimental to viral RNA replication, indicating that ADAR1 plays an essential role in optimal HDV production via RNA editing.

Human immunodeficiency virus type 1. HIV-1 is a retrovirus that causes AIDS and harbors extensive sequence variations, primarily due to recombination and to the lack of fidelity of its reverse transcriptase (49, 55, 81). The observation that ADAR1 mRNA and protein expression increases during HIV infection of primary lymphocytes or lymphocytic cell lines suggests a possible function of ADAR1 during HIV-1 replication (14, 62). Two studies have investigated the role of the editing function of ADAR1 in HIV-1 expression and replication (17, 62). In both studies, overexpression of ADAR1 caused increased virion production of up to 6-fold in HEK 293T cells (17) and between 2- to 10-fold in COS7 cells (62), as measured by HIV-1 p24 in the cell culture supernatants. In addition, siRNAs that reduce the endogenous levels of ADAR1 in HEK 293T cells were also shown to reduce the level of viral protein production (14, 62), further supporting the proviral activity of ADAR1. To determine which role the editing function of ADAR1 plays in this enhancement, the two studies used catalytically inactive mutants but reached different conclusions. In one study, both overexpressed ADAR1, and ADAR1-editing-negative mutant E955A upregulated HIV-1 protein production and increased virion production in HEK 293T cells (17), while in the second study, an editing-negative mutant with mutations in positions 910 to 912 did not enhance virion production in COS7 cells (62). Both mutants were confirmed to lack editing activity, raising the possibility that the discrepancy may be due to differences in cell type.

Edited adenosines in the HIV-1 RNA were found in the 5′ untranslated region and the Tat and Rev sequences and in the vicinity of Rev responsive element (RRE) RNA within the Env gene (17, 62). To evaluate whether ADAR-mediated editing could affect viral replication, an HIV-1 mutant with a 3-nucleotide edited sequence downstream of the RRE was constructed to mimic the naturally edited RNA. This mutant showed enhanced expression compared to that of the wild-type, confirming that the altered sequence increases viral replication (62). In terms of viral infectivity, both virion release and infectivity increased significantly with overexpression of ADAR1, while the catalytic domain mutant did not show this increase, indicating that these properties are regulated by the RNA-editing ability of ADAR1 (17). Interestingly, a recent study showed that ADAR2 also edits HIV-1 RNA and increases viral production and virion release, but in contrast to ADAR1, ADAR2 does not increase the infectious potential of the virus (18). Overall, ADAR1 and ADAR2 appear to enhance HIV-1 replication through an RNA-editing mechanism. In addition, the differences in results suggest that ADAR1 may use an additional mechanism to promote HIV-1 replication.

ADAR1 ENHANCES THE REPLICATION OF RNA VIRUSES VIA PKR INHIBITION

PKR, an IFN-inducible kinase, plays an important role in antiviral immunity, apoptosis, cell proliferation, and stress signaling (24, 25, 68). It is composed of two N-terminal dsRBDs and a C-terminal catalytic kinase domain. Binding of 30- to 50-bp or longer dsRNA or structured ssRNA, such as viral RNA produced during replication, can mediate PKR autophosphorylation and activation. PKR then catalyzes the phosphorylation of the protein synthesis initiation factor eIF2α, leading to inhibition of translation. Recent data show that ADAR1 exerts a proviral activity by binding to PKR via its first dsRBD, followed by inhibition of PKR and eIF2α phosphorylation (14, 52, 84). This effect was demonstrated on MV, VSV, and HIV-1 (14, 17, 43, 52, 80). The mechanism occurs through ADAR1 binding to PKR.

Vesicular stomatitis virus. VSV is a member of the *Rhabdoviridae* family with a negative-sense RNA genome that replicates in the cytoplasm of infected cells. VSV is essentially asymptomatic for humans; however, cattle, horses, and pigs show lesions in the mucous membranes of the mouth and nose, while the virus is neuropathic in mice (87). VSV can exploit defects in the translational regulation of cancer cells, allowing its use as an oncolytic virus (5).

VSV infection is lethal for mice lacking PKR (PKR$^{-/-}$), and PKR$^{-/-}$ murine embryo fibroblasts (MEFs) are more permissive to VSV infection than wild-type fibroblasts (3, 73). The effect of ADAR1 on this virus was assayed in murine NIH 3T3, GP+E86, and wild-type MEF cells, all expressing PKR (52). These cells were more susceptible to VSV infection when they stably expressed ADAR1 p150, as shown by 11-, 32- and 66-fold-higher viral titers in NIH 3T3, GP+E86, and MEF cells, respectively (52). VSV susceptibility was 90-fold higher for MEF PKR$^{-/-}$ cells than for their PKR$^{+/+}$ counterparts, but this increased susceptibility was not increased further by ADAR1, strongly suggesting that this effect is dependent upon PKR expression (52). Variants and mutants of ADAR1 assayed in MEF cells showed that variants missing the Z-DBDs and/or a dsRBD did not increase the level of VSV infection relative to the increase shown with the wild-type ADAR1, whereas a C-terminally-truncated protein in the deaminase domain stimulated VSV infection 60-fold, indicating that this proviral function is independent of RNA editing (52). In contrast, when ADAR1 was stably overexpressed in human HEK 293 cells, VSV growth did not change significantly, suggesting host differences in susceptibility (43). To study the effect of decreased ADAR1 expression on VSV titer, two types of studies were performed. First, transient silencing of endogenous ADAR1 with siRNA in VSV-infected HEK 293T cells reduced

the viral titer 8-fold compared to that for the control, making these cells more resistant to VSV infection (52). Second, HeLa cell lines in which ADAR1 expression was stably knocked down by RNAi (ADAR1^{kd}) were constructed (80) and used to measure VSV titer (43). ADAR1^{kd} cells expressed less than 15% of the amount of ADAR1 p110 and less than 10% of the amount of ADAR1 p150 expressed by the parental cell lines (43, 80). When these cells were treated with beta interferon (IFN-β), the knockdown of ADAR1 decreased the VSV titer about 10-fold more than IFN inhibition but had no effect on nontreated cells (43). While the knockdown of ADAR1 had no effect on the total levels of PKR, it increased PKR and eIF2α phosphorylation in cells treated with IFN (43). Overall, both studies showed an enhancement of VSV replication mediated by ADAR1 that was independent of its RNA-editing activity and dependent on its inhibitory effect on PKR activation.

Measles virus. MV is a negative-stranded RNA virus that is a member of the *Paramyxoviridae* family. It replicates in the cytoplasm of the host cell and causes an acute respiratory illness in humans (65, 89). A persistent infection of the central nervous system can also occur in rare cases, leading to a fatal neurological disease known as subacute sclerosing panencephalitis (SSPE), which is correlated with an ADAR-mediated hyperediting activity in MV sequences (2, 13, 37). The MV vaccine (MVvac) strains are devoid of SSPE consequences and show selectivity against tumor cells, which indicates their therapeutic potential as oncolytic viruses (21).

To study the influence of ADAR1 on the growth of MV, HeLa ADAR1^{kd} cells were used (80). These cells and ADAR1-sufficient HeLa cells were infected with a modified MVvac carrying the green fluorescent protein (GFP) gene. While there was a high level of GFP reporter signals present in the parental ADAR1-sufficient cell line, the GFP signal from an MV virus deficient for the C gene, a virulence factor, was 30-fold lower in HeLa ADAR1^{kd} cells, suggesting that ADAR1 enhances the replication of the virus (80). MV is a cytolytic virus that induces cell death by apoptosis mediated by PKR activation (79). In HeLa ADAR1^{kd} cells, apoptosis induced by the wild-type virus and MV deficient for the V gene, another virulence factor, was enhanced by 30% and 40%, respectively. Consistent with these observations, PKR phosphorylation was increased after MV infection with the three MV variants. In contrast, very low PKR activation levels were detected following MV infection of HeLa ADAR1/PKR-sufficient cells (80). Activation of PKR in infected HeLa ADAR1^{kd} cells also correlated with increased phosphorylation of eIF2α and interferon regulatory factor 3 (IRF-3). These observations point out an antiapoptotic and proviral role for ADAR1 in MV infection through the suppression of dsRNA-dependent pathways such as PKR and IRF-3.

HIV-1. In addition to the effect of ADAR1 on HIV-1 replication via RNA editing, an effect via PKR inhibition was also characterized. In lymphocytic Jurkat T cells infected with HIV-1, PKR was transiently activated, but this activation was reversed when virus production was high enough to be visible by immunoblotting against the capsid protein (14). This reduction in PKR phosphorylation also correlated with an increase in the ADAR1 p150 and p110 forms in the cells. In addition, PKR-ADAR1 interactions dramatically increased at the peak of HIV-1 production compared to those in noninfected cells

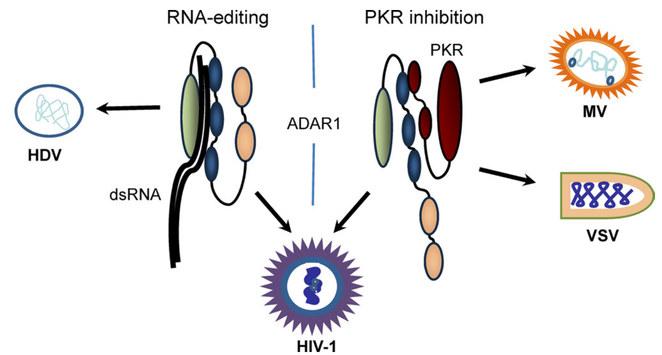

FIG. 2. ADAR1 enhancement of the replication of RNA viruses via RNA editing and inhibition of PKR. (Left) ADAR1 enhances the replication of HDV and HIV-1 via RNA editing. (Right) ADAR1 enhances the replication of MV, VSV, and HIV-1 via the inhibition of PKR.

(14). The overexpression of ADAR1 in HEK 293T cells increased HIV-1 production 2- to 8-fold in the absence of exogenous PKR and completely rescued PKR-induced inhibition of HIV-1 production. This increased viral production correlated with decreased phosphorylation of PKR and eIF2α, and ADAR1 counteracted the inhibitory effect of PKR (14, 17). A similar, but less pronounced, effect on eIF2α phosphorylation was also observed with ADAR2 (18). ADAR1 proteins mutated or deleted in the catalytic deaminase domain were still able to increase HIV-1 production, whereas the absence of the dsRBDs was deleterious for this effect, showing that this activity was independent of RNA editing (14, 17). Furthermore, in astrocytic cells that inadequately support HIV-1 replication due to a heightened PKR response, ADAR1 increased HIV-1 production in a manner similar to that of another PKR inhibitor, the TAR RNA binding protein (TRBP) (14, 56). These results strongly suggest that ADAR1 proviral activity on HIV-1 is also mediated in large part by PKR inhibition (Fig. 2).

PKR inhibition by ADAR1 in the context of these viruses raised a question about the mechanism of interaction between these two proteins. Immunoprecipitation assays with ADAR1 and PKR in the presence of RNase A and V1 showed a direct protein-protein interaction only when the first dsRBD of ADAR1 was present (52). Furthermore, a two-hybrid screen using PKR as bait isolated ADAR1 domains, all of which had the first dsRBD, thereby confirming the interaction and a likely inhibition through this domain (14).

CONCLUSIONS

ADAR1 has a new role in enhancing viral replication. Because ADAR1 is synthesized from an IFN-stimulated gene, it would appear obvious that the protein should contribute to the cell innate immune pathway that would react against virus replication (29, 64, 70). In addition, the similarity in function with APOBEC3G, a DNA cytidine deaminase acting against HIV-1 in the absence of the Vif protein, would further suggest an antiviral role (1, 74). Although this hypothesis may be true for some viruses, in the case of the RNA viruses described here, HDV, HIV-1, VSV, and MV, it has now been clearly

shown that ADAR1 enhances viral replication via RNA editing and inhibition of PKR (Table 1 and Fig. 2). For HIV-1, both mechanisms act in concert to increase viral replication (14, 17, 62). Because of the biological significance of ADARs in editing cellular miRNAs and by inhibiting RNAi function independently of editing, ADARs could also have an impact on viral replication by editing and modifying the function of viral miRNAs (32, 35, 53, 54, 71, 91). Indeed, this function has been shown to enhance the replication of Epstein-Barr virus and human herpesvirus 8, two herpesviruses, but has not been demonstrated thus far for RNA viruses (23, 36). Considering that HIV-1 carries viral siRNAs (7, 41, 58) and that VSV replication is downregulated by cellular miRNAs (57), further research may reveal if ADAR1 can edit them and modify their function.

ADAR1 enhances or prevents viral replication in various cellular contexts. Because every biological function is the result of a balance among different activities, the above findings can be complemented by observations that for some viruses, ADAR1 could also have dual roles, both proviral and antiviral (69). Indeed, viral RNA editing by ADARs increases sequence diversity that might be favorably selected in a specific cellular context but might also be deleterious when the sequences are hyperedited. HDV, for example, is typical of a virus that evolved with the optimal amount of ADAR1, which becomes deleterious for the virus when increased or decreased (38, 39). The ADAR1 editing activity in MV also reflects this complexity by exhibiting various results in different cells. The rare cases of human SSPE have been correlated with hyperediting of several MV genes in human brain cells, which leads to a persistent, less fitted but more pathogenic virus, suggesting an antiviral role for ADAR1 (2, 12, 13, 60, 75). Increased MV replication has been observed in murine embryonic cells with a deletion of ADAR1 p150 expression (86); however, this effect was not correlated to a decrease in editing compared to that of the control. These cells are derived from nonviable embryos, and the increased replication could be due to other disturbed mechanisms. In contrast, ADAR1 knockdown in human cells induced a decrease in MV replication attributed to an absence of PKR inhibition, indicating an enhancement of viral replication mediated by ADAR1 (80).

ADAR1 has a new function in modulating the function of another IFN-induced protein. For VSV, HIV-1, and MV, ADAR1 inhibits PKR-induced apoptosis mediated by cellular stresses and viruses and therefore contributes to a high level of virus replication (14, 16, 17, 43, 52, 80). While it seems unexpected that an IFN-induced protein would counteract the IFN response by specific inhibition, this finding correlates with and explains part of the observed general downregulation of the innate immune pathway mediated by ADAR1 (30, 85). The similarity with another autoregulatory loop between miRNA-103/107 and Dicer, an endonuclease which is itself involved in the maturation of miRNAs, emphasizes that multiregulation of cellular processes is necessary to maintain cell function (47). The autoregulation between two IFN-induced proteins during viral infection emphasizes that the cell response is not intended to counteract viral replication but is the result of the concomitant evolution of viruses with that of their hosts. While PKR is regulated by a large number of cellular and viral RNAs and proteins, only a few factors have been shown to regulate ADAR1 activity (15, 25, 26, 29, 64). This novel type of proviral regulation by an IFN-induced protein changes and complements our understanding of the innate immune response. This response may not always be detrimental to the virus because cells need to reach an equilibrium to avoid death, due to either viral replication or to cell apoptosis induced by a hyperactive antiviral response. In the case of HIV-1, the manipulation of the host response by the virus seems to lead to both virus replication and cell survival, but this equilibrium is unstable and ultimately leads to chronic inflammation of lymphoid cells, which contributes to disease progression (8, 15). For many lytic viral infections, the extent of cell death via viral replication and a hyperactive host immune response could determine the extent of cell survival and disease remission and could also be exploited for increased oncolysis of tumor cells (4, 21, 24–26).

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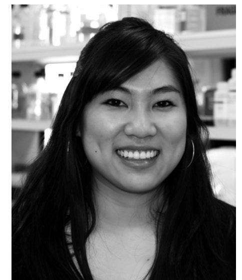

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