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Protein Kinase PKR and RNA Adenosine Deaminase ADAR1: New Roles for Old Players as Modulators of the Interferon Response

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Abstract

Double-stranded RNA (dsRNA) plays a centrally important role in antiviral innate immunity, both for the production of interferon (IFN) and also in the actions of IFN. Among the IFN inducible gene products are the protein kinase regulated by RNA (PKR) and the adenosine deaminase acting on RNA (ADAR1). PKR is an established key player in the antiviral actions of IFN, through dsRNA-dependent activation and subsequent phosphorylation of protein synthesis initiation factor eIF2α thereby altering the translational pattern in cells. In addition, PKR plays an important role as a positive effector that amplifies the production of IFN. ADAR1 catalyzes the deamination of adenosine in RNA with double-stranded character, leading to the destablization of RNA duplex structures and genetic recoding. By contrast to the antiviral and proapoptotic functions associated with PKR, the actions of ADAR1 in some instances are proviral and cell protective as ADAR1 functions as a suppressor of dsRNA-mediated antiviral responses including activation of PKR and interferon regulatory factor 3.

Introduction

Interferon (IFN), the first cytokine discovered [1], derives its name from the robust biological activity for which it was discovered: the ability to *interfere* with virus growth. Interferon represents the founding cornerstone of antiviral innate immunity. Double-stranded RNA (dsRNA) has a long history in the interferon field. DsRNAs, both naturally occurring and synthetic, exemplified by reovirus genome RNA and poly rI: poly rC, respectively, were identified decades ago as potent inducers of IFN [2–4]. We now have significant understanding of the molecular mechanisms by which dsRNA produced during viral infections is detected as foreign by cellular nucleic acid sensors, thereby triggering signal transduction pathways that culminate in the transcriptional activation of IFN genes [5–8]. As shown schematically (Fig. 1, *left*), sensors of dsRNA in pathogen infected cells include the RIG-like family of receptors (RIG-I, MDA5) present in the cytosol [6,7] and the Toll-like receptor TLR3 that is endosomal membrane-associated [5]. RNA polymerase III also acts as a cytosolic sensor of DNA, leading to the production of dsRNA that is sensed by the RIG pathway [8–10].

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The actions of IFNs are mediated by induced gene products and this too can involve dsRNA [11,12]. Transcriptional activation of IFN-stimulated gene (ISG) expression by the canonical JAK-STAT signaling pathway is illustrated in Figure 1 (*right*). Type I and III IFNs bind to their cognate receptors and activate a trimeric factor complex composed of STAT1 and 2 and IRF9 that then translocates to the nucleus and binds the DNA enhancer known as the interferon-stimulated response element (ISRE). Among the IFN inducible gene products are the PKR protein kinase [13–16] and the ADAR1 RNA adenosine deaminase [17–19]. Both PKR and ADAR1 are dsRNA binding proteins that possess multiple copies of a conserved dsRNA binding motif (Fig. 2A). In the case of PKR, dsRNA is a regulatory effector that either activates or antagonizes kinase activity; in the case of ADAR1, dsRNA is typically the substrate of the deaminase. Recent evidence suggests that PKR and ADAR1 function not only as mediators of the actions of IFN, but that they may also play roles in the production of IFN.

Protein Organization and Genetic Regulation of ADAR1

ADAR1, adenosine deaminase acting on RNA 1, catalyzes the C6 deamination of adenosine to produce inosine in RNA substrates with double-stranded (ds) character [14,18,20,21]. This reaction (Fig. 2B) is referred to as A-to-I RNA editing and is a form of nucleotide substitution editing. The generated I is decoded as G instead of A by ribosomes during translation and by viral polymerases during RNA-dependent RNA replication. A-to-I editing also alters the stability of RNA duplex structures because I:U mismatch base pairs are less stable than A:U base pairs [22–24]. Indeed, ADAR activity was first described as a dsRNA duplex unwinding activity, but instead of unwinding dsRNA, the deamination of adenosine in duplexes destabilized the dsRNA structure [18,25,26].

The ADAR1 deaminase is encoded by a single gene [14,18]. The major transcript seen in human cells is ~6.7-kb in size, is increased in steady-state amount by IFN treatment, and includes 17 exons and possesses an open reading frame (ORF) of 1226 amino acids [14,17,18]. Two differently sized ADAR1 proteins are expressed, an IFN inducible (p150) protein that is found in both the cytoplasm and nucleus and a constitutively expressed (p110) protein that is predominantly if not exclusively localized to the nucleus [17,18,27]. ADAR1 transcription is driven by multiple promoters: one is IFN inducible, and the others constitutively active [18,28,29]. The IFN inducible promoter possesses a consensus ISRE element; activation following type I IFN treatment is dependent upon the IFNAR receptor, JAK1 kinase and STAT2 factor, but surprisingly not on STAT1 [28–30]. ADAR1 transcripts undergo alternative splicing involving exons 1 and 7 to encode the inducible p150 protein (1200 amino acids) or the constitutively expressed p110 protein (931 amino acids). p110 is an N-terminally truncated version of p150 (Fig. 2A).

The domain structure of the ADAR1 proteins includes within the C-terminal region the deaminase catalytic domain, and within the N-terminal region two kinds of nucleic acid binding domains, for dsRNA and for Z-DNA (Fig. 2A). Three copies of the dsRNA binding domain (R_I, R_{II}, R_{III}) are found in both p150 and p110; they are similar to the prototypical dsRNA binding R-domain (dsRBM) first identified in PKR [31]. Two Z-DNA binding domains (Zα, Zβ) are found in p150, but only Zβ is present in p110 [14,17,18,32]. p150 also includes a nuclear export signal [33]. Mutational analyses have established the importance of key residues in the ADAR1 functional domains, including the H910Q, E912A double mutant that inactivates deaminase catalytic activity; mutations of a conserved lysine at positions K554, K665 and K777 that affect RNA binding activity; the K418R mutation that abolishes sumoylation; and, mutations including Y177F that affect Z-DNA binding activity [14,34–36].

A-to-I editing is of two general types [19,20,24,25]. Editing can be highly site-selective with deamination occurring at one or very few specific A's in the RNA substrate such as is seen with the glutamate and serotonin receptor pre-mRNAs and the hepatitis delta virus (HDV) antigenome RNA. These selective edits alter the genetic decoding of mRNA during translation, as the I pairs with C, whereas A pairs with U. A-to-I editing can also occur at multiple A's in RNA substrates with near perfect duplex structure, such as observed when ADAR activity was discovered [18,25,26] or with synthetic dsRNA or viral dsRNA [14,18]. Hence, RNA substrate selectivity for adenosine deamination ranges from highly site-selective to non-selective, and is dependent in part on the duplex structure of the substrate RNA and possibly also interacting protein partners [18]. Little information is available regarding RNA as an effector rather than substrate, although adenovirus VAI RNA does antagonize ADAR1 editing activity [18,26]. A-to-I editing can affect how viruses interact with their hosts, either directly through genetic recoding, or indirectly through perturbations of RNA structures qualitatively if not quantitatively [26]. The importance of ADAR1 proteins to mammals furthermore is revealed by the embryonic lethality seen following genetic disruption of *Adar1* expression. Independent *Adar1* disruptions that knockout both p150 and p110 expression [37–40] or only p150 expression [41] all display embryonic lethality.

Protein Organization and Genetic Regulation of PKR

The IFN inducible, dsRNA-activated protein kinase (PKR, also known as eukaryotic translation initiation factor 2-alpha kinase 2 [EIF2AK2], P1 kinase, or p68 kinase) is a member of the eIF2α family of protein kinases that also include the PKR-like endoplasmic reticulum kinase (PERK, or EIF2AK3); the general control non-derepressible 2 kinase (GCN2, or EIF2AK4); and the hemin-regulated inhibitor (HRI, or EIF2AK1) of translation [42,43]. These eIF2α kinases are activated under different conditions of cellular stress, which is viral infection in the case of PKR, and catalyze the phosphorylation of serine 51 of eIF2α [42]. PKR expression is inducible by IFN [13]; the *Pkr* gene promoter, like the inducible ADAR1 promoter, possesses a consensus ISRE element [14,16]. The major PKR gene transcript seen in human cells includes 17 exons and specifies an ORF of 551 amino acids [14–16]. In contrast to the embryonic lethality observed by genetic disruption of mouse *Adar1*, neither of the two differently targeted *Pkr* homozygous null disruptions is lethal [44,45].

Two RNA binding motifs (R_I, R_{II}) are present within the N-terminal portion of PKR; the C-terminal region includes the kinase catalytic domain [14,16,46] as shown by Figure 2A. Mutational analyses established the importance of the PKR functional domains, including the K296R mutation in the catalytic subdomain II that impairs kinase activity; mutations of the highly conserved lysine of the RNA-binding domain (K64 in R_I) that impair RNA binding activity; and the T446A phosphorylation site mutant that impairs *in vivo* kinase activity [12,14,15]. Binding of dsRNA leads to a PKR conformational change —which is believed to dissociate the catalytic domain from the autoinhibitory N-terminal domain— permitting dimerization and autophosphorylation [14,15,47]. RNA-mediated activation of PKR involves phosphorylation of both serine and threonine sites [14], including the T446 residue within the catalytic region that is commonly used as a measure of activation. Activation of PKR also has been described to include phosphorylation of tyrosine residues [48]. In addition to synthetic and natural duplex RNAs that activate PKR, naturally occurring viral RNAs with double-stranded character function either as PKR activators or antagonists as illustrated by reovirus s1 mRNA and adenovirus VAI RNA, respectively [12,14,49]. RNA binding is believed to occur in a non-sequence specific but RNA structure-dependent manner [11,12,50].

PKR Acts to Amplify Induction of Interferon following Virus Infection

PKR has long been known as a key player in the actions of IFNs [11,12]. Evidence is accumulating that PKR also plays an important role in the induction of type I IFNs, particularly IFNβ during viral infections [16,44,51–53]. The cytoplasmic RIG-I like receptors (RLRs) are principal sensors of foreign or non-self viral RNAs (Fig. 1, *left*). A complex forms involving the mitochondrial adaptor IPS-1 (also known as VISA, CARDIF, or MAVS) and activated RLRs, and together with TRAFs and IKK-related kinases, signal the activation of IRF3 and NFκB [5–7] to drive IFNβ gene expression [54].

Recent studies show that activation of PKR accompanies IRF3 activation, and that knock-down of PKR reduces activated IRF3 levels and IFNβ induction by transfected dsRNA [55]. Furthermore, the effect of PKR on activation of IRF3 is dependent on the adaptor IPS-1; siRNA mediated knock-down of either IPS-1 or PKR reduces IFNβ gene expression in response to measles virus (MV) infection to similar levels [51]. It is not yet known whether PKR directly phosphorylates IRF3, or indirectly contributes to the assembly or activation of the RIG-I/IPS-1 dependent signaling complex. For several positive-stranded RNA viruses including West Nile virus (WNV), encephalomyocarditis virus (EMCV), Theiler's murine encephalomyelitis virus and Semliki Forest virus, PKR also is required for production of IFNβ [52, 53]. But for JFH1 hepatitis C virus (HCV), which likewise is a positive-stranded RNA virus, phosphorylation of PKR and eIF2α leads to an inhibition of IFNβ induction at the level of translation [56]. HCV, whose IRES-mediated protein synthesis initiation is independent of eIF2α phosphorylation, thus utilizes PKR to inhibit both the production [56] and action [57] of IFN. For bovine rotavirus UK, a double-stranded RNA virus, optimal induction of IFNβ production also is dependent upon PKR as well as RLRs, IPS-1, and IRF3; PKR deficiency leads to a defect in UK-infected cell secretion of IFNβ [58]. The mechanisms are not yet resolved, but one possibility in EMCV-infected cells involves PKR-mediated regulation of IFN mRNA stability [52].

The PKR dependency for optimal IFNβ induction by MV correlates with enhanced activation of NFκB and ATF2, and with WNV the PKR effect also involves enhanced NFκB activation [53]. The possibility that the PKR amplification of IFNβ expression is a translational control effect through eIF2α phosphorylation cannot be excluded, for example affecting the level of an activated transcription factor such as NFκB through control of synthesis of a rapidly degraded inhibitor like IκBα. To the extent that RNA structural features overlap for recognition by the RLRs and PKR also is not yet clear, but length of dsRNA and presence of a 5′-triphosphate on ssRNA are important for RLR sensing [5,59]. However, studies with reovirus ssRNA transcripts and genome dsRNA indicate that a 5′-triphospate is neither necessary nor sufficient for PKR activation [12,60], although 5′-triphosphate dependent activation of PKR by synthetic RNAs with short stem loops has been reported [61]. Interaction of PKR with members of the TRAF protein family has been described, with TRAF2 and TRAF3 interacting sites located around R_{II} [62]. These interactions possibly stabilize the dsRNA-induced active (open) conformation of PKR by preventing the autoinhibitory function of R_{II}. Furthermore, TRAF5 and 6 have been shown to interact with PKR, presumably indirectly and mediated by TRAF3 and 2, respectively [63]. The assembly of a TRAF-platform might enable the formation of an alternative signaling complex leading to phosphorylation of IRF3 or conceivably mediate an interaction with the RIG-I/IPS-1 signaling complex, thereby enhancing NFκB activation. Activation of NFκB by recruitment of the IKK complex [64], consisting of the inhibitor of kappaB kinases IKKα, IKKβ and IKKγ (also known as NEMO), could also be mediated by TRAFs. The IFNβ enhanceosome, besides NFκB and IRF3, also consists of ATF2/c-Jun activated by the mitogen-activated protein kinase (MAPK) pathways, including p38 and Erk1/2. PKR has been shown to be involved in the activation of p38 and Erk1/2 [51,65], but the biochemical mechanisms underlying this activation are not yet fully resolved.

ADAR1 Acts as a Suppressor of PKR and Displays Proviral Properties

Although ADAR1 is an IFN-inducible gene product, a growing body of evidence shows that ADAR1 functions in a proviral manner during acute infection in cell culture, notably with some RNA viruses [19,66]. These include HDV [67], MV [68], vesicular stomatitis virus (VSV) [27,69] and human immunodeficiency virus (HIV-1) [70–73]. In the case of HDV, site-selective A-to-I editing changes an amber UAG termination codon to a tryptophan UIG codon that permits synthesis of large delta antigen [67]. In the case of MV acute infection, depletion of ADAR1 in MEFs by genetic knockout of p150 and in human cells by knockdown of both p110 and p150 results in enhanced apoptosis and virus-induced cytotoxicity, and in the human ADAR1-deficient cells reduced MV growth [41,68]. In the case of HIV-1, overexpression of either ADAR1 [70,71,73] or ADAR2 [72] increases viral replication by both editing-dependent and-independent mechanisms.

While multiple mechanisms may be responsible for the proviral activity of ADAR1, one relates to the inhibition of PKR [74]. ADAR1 interferes with the activation of PKR and reduces the phosphorylation of eIF2α [27,68,69,75]. Overexpression of ADAR1, either the full-length p150 protein or the region with the RNA-binding and Z-DNA binding domains alone, impairs both PKR autophosphorylation and eIF2α phosphorylation [69,73,75]. Perhaps more physiologically relevant than overexpression are loss of function studies, where stable knockdown of ADAR1 leads to enhanced PKR autophosphorylation and eIF2α phosphorylation following infection with C^{ko} mutant MV or VSV [27,68]. That is, in ADAR1 sufficient cells, PKR autophosphorylation is suppressed following infection, but in ADAR1 deficient cells is enhanced possibly because of the lack of editing-mediated destablization of dsRNA, lack of sequestration of dsRNA by ADAR1, or lack of formation of inactive heterodimeric ADAR:PKR complexes.

By virtue of the ability to impair PKR activation, ADAR1 would be expected to suppress PKR-mediated biological activities including antiviral, proapoptotic, and IFN induction amplification activities. Some of these predictions have been tested, either by ADAR1 loss of function or overexpression strategies. Depletion of ADAR1 by knockdown in human cells or by genetic knockout in mouse MEFs leads to enhanced apoptosis and cell cytotoxicity following infection with a number of different viruses of the *Paramyxoviridae* families and the DNA virus, polyoma [41,68,76]. In the case of MV, growth of both wild-type and V^{ko} mutant virus are reduced in ADAR1 deficient cells compared to ADAR1 sufficient cells [68]. Furthermore, the inhibition of VSV growth by IFN is about 1 $\log_{10}$ further reduced in ADAR1 deficient compared to sufficient cells, and the reduced VSV yield correlates with enhanced PKR activation [27]. Using an overexpression screening strategy in which more than 380 human ISGs were tested for their antiviral activity against a number of medically important viruses, different categories of ISGs were identified: some acted broadly with an inhibitory effect, and a few enhanced viral replication [77]. Among the most potent *proviral* ISGs was ADAR1 that significantly enhanced the replication of all viruses tested including HIV-1, WNV, chikungunya virus, Venezuelan equine encephalitis virus and yellow fever virus [77].

Finally, for some RNA viruses including human respiratory syncytial virus and lymphocytic choriomeningitis virus, isolates have been obtained with A-to-G and U-to-C nucleotide substitutions in viral glycoproteins, sequence changes consistent with editing by ADAR1 [19]. Conceivably, limited low level editing by ADAR1 might be proviral in an infected animal if changes in a surface antigen occur in a manner that alters an epitope structure sufficient to allow escape from immune surveillance and neutralization. By contrast, when extensive editing occurs leading to hypermutations and inhibition of protein production, whether the editing is by ADAR1, or APOBEC3G in the case of retroviruses, then the effect could be antiviral.

Inosine-containing RNA as an Effector of the Innate Immune Response: Activator or Suppressor?

Poly rI:poly rC was one of the first dsRNA inducers of IFN discovered [3], and remains one of the most potent and efficient inducers [5–7]. Poly rI: poly rC also is bound by PKR, and dependent upon the concentration, either activates or inhibits of PKR autophosphorylation as well as activation of the 2′,5′-oligoadenylate synthetases [11,12,42]. ADAR1 action on dsRNA produces inosine (I) from adenosine (A) (Fig. 2B). Thus, A-to-I editing of dsRNA would be expected to produce inosine-containing dsRNA with I:U pairs in place of A:U pairs. Synthetic dsRNA with I:U base pairs unexpectedly did not induce, but rather suppressed the IFN response [78]. Induction of IFN-stimulated gene expression and apoptosis by poly rI: poly rC was suppressed by IU-dsRNA that contained multiple I:U pairs [78]. Furthermore, the IRF3 activation was inhibited by IU-dsRNA, possibly by inhibition of RIG-like receptor signaling, although the precise mechanism remains unresolved [78]. The observation that synthetic IU-dsRNA inhibits IRF3 activation is consistent with prior studies with virus-infected cells. Infection of ADAR1-deficient cells with MV results in an enhanced activation of IRF3 compared to the activation seen in ADAR1-sufficent cells [68].

The p150 isoform of ADAR1 has emerged as an important component in the host response to infection by a number of RNA viruses that replicate either in the cytoplasm or nucleus [27,41,68], whereas the p110 isoform plays a role for some DNA viruses that replicate in the nucleus [76]. A common theme is that the presence of ADAR1 correlates with a cell protective response, and in some cases even enhances virus replication. By contrast, under conditions of ADAR1 deficiency either in genetically null MEFs or knockdown cells, PKR activation is increased and the replication of VSV [27,69] and MV [68] are reduced. These results further establish that ADAR1 can display antiapoptotic and even proviral behavior in cell culture. Additionally, foreign DNA present in the cytosol, like foreign RNA, can trigger an innate immune response [8,79]. Among the DNA sensors in addition to RNA polymerase III [8] is DAI, DNA-dependent activator of IFN [79]. ADAR1 strongly suppresses the activity of DAI and reduces IFNβ induction by herpes simplex virus infection, effects that might enhance virus replication.

ADAR1 and Development of the Hematopoietic system

ADAR1 plays an important role in the development of the immune system. The presence of ADAR1 protein is an obligate requirement in mice for development of the liver and bone marrow hematopoietic system as established by knock-out studies. In mice homozygous for the *Adar1* null mutation, embryonic lethality occurs at day 11.5–12.5 with liver disintegration and widespread apoptosis in many tissues [37]. MEFs from *Adar1*$^{-/-}$ embryos deficient in both p110 and p150 are prone to apoptosis due to stress induced by serum deprivation [39], and MEFs deficient in only p150 show enhanced cytotoxicity following viral infection [41]. Furthermore, the absence of ADAR1 p110 and p150 proteins results in a global upregulation of type I and II IFN-inducible transcripts and apoptosis [38], consistent with analyses that show ADAR1 suppresses the IFN response [27,68,69]. Studies of inducible *Adar1* deficient mouse lines indicate that ADAR1 suppresses the deleterious effects of a robust activation of the IFN response [38]. ADAR1 either protects hematopoietic stem cells from apoptosis [38] or is necessary for differentiation of hematopoietic progenitor cells [40]. The selective knockout of p150 [41], like the knockouts that disrupt both p110 and p150 [37–40], is embryonic lethal, suggesting that the IFN inducible p150 isoform of ADAR1 is the form that regulates IFN production and protects against stress-induced cytotoxicity, thereby facilitating cell survival and maintenance of the hematopoietic stem cells.

Stress, RNA Granules, PKR and ADAR

Stress granules (SG), a form of RNA granules found in the cytoplasm of cells, can be both a cause and a consequence of stress-induced alteration in translation [80]. SG form within cells during conditions of stress, including viral infection, and among the consequences are the global downregulation of translation and the production of proteins necessary for cell survival [81,82]. Multiple mechanisms may be involved in the SG-associated modulation of translation, and among them is the phosphorylation of eIF2α. Formation of SG during viral infection often, but not always, is associated with activation of PKR and phosphorylation of eIF2 [80–82]. While some viruses mediate PKR activation and eIF2α phosphorylation, other viruses encode gene products that antagonize these processes [12,83].

Vaccinia virus E3L protein antagonizes PKR activation and facilitates virus growth, but mutant virus lacking E3L grows poorly, activates PKR and eIF2α phosphorylation and induces the formation of cytoplasmic SG-like structures [84,85]. Effective PKR-mediated restriction of E3L mutant virus growth requires SG-like complex formation subsequent to eIF2α phosphorylation [84]. Mammalian reovirus also induces the formation of SG in an eIF2α phosphorylation dependent manner early during infection, but at later times the SG structures become disrupted which correlates with the release of viral, but not cellular, mRNA from translation inhibition [86]. PKR activation and eIF2α phosphorylation also are associated with induction of SG formation by respiratory syncytial virus, but in a manner that facilitates virus replication [87]. In the case of WNV and dengue virus, the NS3 protein and viral dsRNA co-localize with the SG components TIA-1 and TIAR, and interaction with TIAR facilitates genome RNA synthesis and inhibits SG formation [88].

Among the SG-associated proteins is ADAR1 [80]. Furthermore, synthetic IU-dsRNA associates with a SG-like complex and downregulates gene expression in cultured cells [89]. Over-expression of ADAR1 increases gene expression at the translational level by decreasing PKR-dependent eIF2α phosphorylation [75]. What is not yet clear is whether the overexpression of ADAR1 simply impairs PKR activation, or alternatively alters SG-formation and function, to affect gene expression. It also is not yet known whether naturally occurring I-containing RNAs, similar to the synthetic IU-dsRNAs, or cytosolic RNA sensor components of the RIG-like receptor-IPS signaling complex, also associate with SG-like complexes to modulate gene expression and the innate immune response.

Conclusions

PKR, an IFN-inducible protein, is firmly established as a regulator of translation in virus-infected cells through phosphorylation of protein synthesis initiation factor eIF2α. Increasing evidence further positions PKR as a positive effector of IFN production triggered by infection and elicited via the RIG-like receptor pathway. PKR-mediated amplification of IFNβ expression is described for several RNA viruses, although there is the counter exception illustrated by HCV that displays a PKR-dependent impairment of IFNβ production. For PKR-dependent IFNβ expression, increased phosphorylation of PKR correlates with increased activation of IRF3, NFκB and ATF2, and enhanced IFN induction.

ADAR1, likewise is an IFN-inducible protein, is best known for its A-to-I RNA editing activity, whereby adenosine in dsRNA structures is deaminated to produce inosine. Increasing evidence implicates ADAR1 as an important modulator of the innate antiviral response, down-regulating the IFN response. ADAR1 suppresses activation of both PKR and IRF3, for example. In addition to the effects of A-to-I editing on RNA structure and function, ADAR1 also may conceivably affect the innate immune response via mechanisms that are dependent upon the RNA-binding or protein-interaction properties of ADAR1. However, little is known about the catalytic-independent mechanisms of ADAR1 in

mammalian cells. Likewise, the precise mechanism as to how ADAR1 impairs PKR and IRF3 activation is not resolved. Among the possibilities are the destabilization of dsRNA structures; dsRNA competition and sequestration; and altered protein-protein interactions. Nor is the mechanism resolved as to how PKR affects signaling to amplify IFNβ production, perhaps either by translational inhibition of suppressor proteins or by fulfilling an adaptor function during signaling. These remain important questions.

There are an increasing number of cellular sensors of foreign nucleic acids that have been identified which trigger the innate antiviral response, including RLRs and TLR3 for dsRNA. PKR and ADAR1 also are sensors of dsRNA (Fig. 3). Although both are IFN inducible proteins, constitutive levels of PKR and ADAR1 are typically present in most mammalian cells. A considerable body of evidence characterizes PKR function as antiviral and proapoptotic, whereas a growing body of evidence reveals ADAR1 function typically as proviral and antiapoptotic. A model that emerges is characterized by the actions of ADAR serving to balance those of PKR and other innate immune system responders (Fig. 3). Under spatiotemporal cellular conditions of ADAR sufficiency and PKR deficiency, PKR and IRF3 activities, for example, are minimized through functional inactivation of dsRNA by editing or sequestration. By contrast, under conditions of ADAR deficiency and PKR sufficiency, the activities of PKR and IRF3 are maximized. Future studies of the ADAR and PKR will provide opportunities to further test these notions, and no doubt will continue to provide us with surprises and new insights into biological functions of these IFN-inducible, dsRNA-binding enzymes.

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Abbreviations

ADAR	adenosine deaminase acting on RNA
dsRNA	double-stranded RNA
IFN	interferon
PKR	protein kinase regulated by RNA
ssRNA	single-stranded RNA

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Highlights

- PKR and ADAR1 as dsRNA sensors.
- ADAR1 and PKR as opposing modulators of the interferon response.
- ADAR1 suppresses PKR and IFNβ induction.
- Proviral and cell protective functions of ADAR1.
- Antiviral and Proapoptotic functions of PKR.

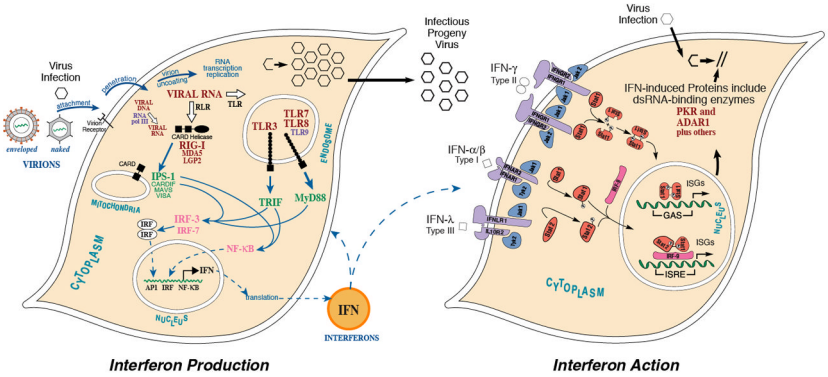
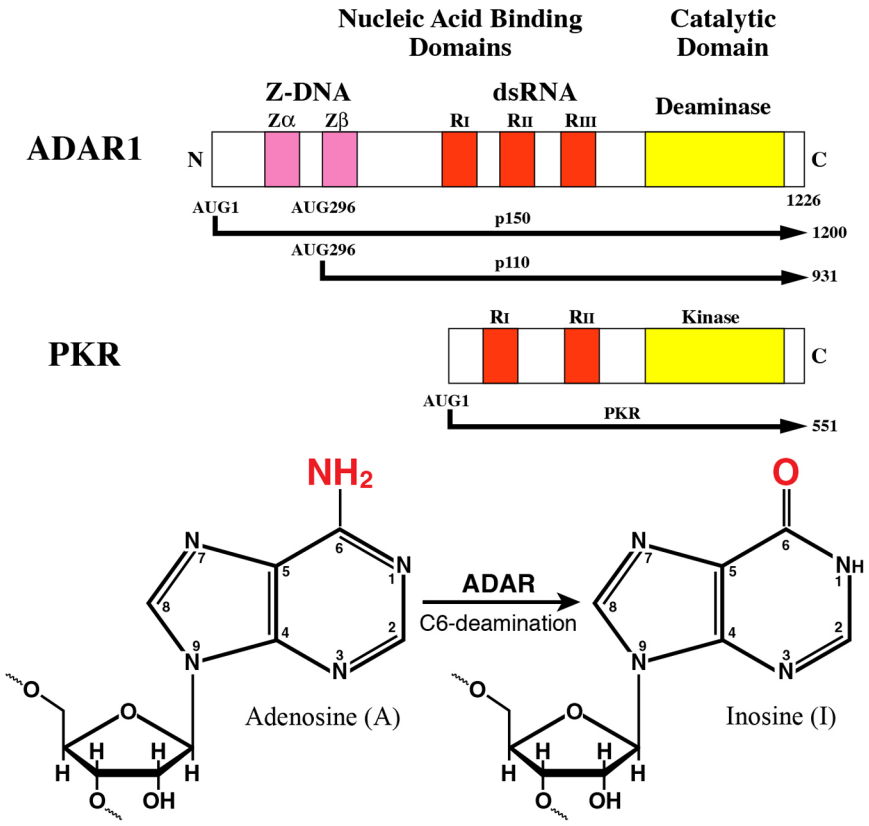


Figure 1. Signaling pathways involved in the induction and action of interferon

(left) Nucleic acid sensors that respond to viral infection detect viral nucleic acids as foreign, thereby leading to the production and action of IFN. Sensors include the RIG-I and MDA5 cytosolic helicases and their mitochondrial membrane-associated adaptor IPS-1; the Toll-like receptor TLR3 that acts through the TRIF adaptor; TLRs 7, 8 and 9 that act through the MyD88 adaptor; and, RNA polymerase III that acts through IPS-1. RIG-I, MDA5 and TLR3 sense dsRNA, and pol III senses cytosolic dsDNA to produce dsRNA. TLRs 7 and 8 sense ssRNA, and TLR9 CpG-rich DNA. These nucleic acid sensors trigger antiviral innate immunity through activation of factors that lead to transcriptional activation of IFN production. *(right)* Signaling by types I, II and III interferons through the canonical JAK-STAT pathway is illustrated, leading to the transcriptional activation of IFN-stimulated genes. Among the IFN-induced gene products are ADAR1 and PKR, both of which bind dsRNA and possess enzymatic activity, one (PKR) regulated by dsRNA and the other (ADAR1) utilizing dsRNA as a substrate. *Adapted from Samuel [*26*]*.



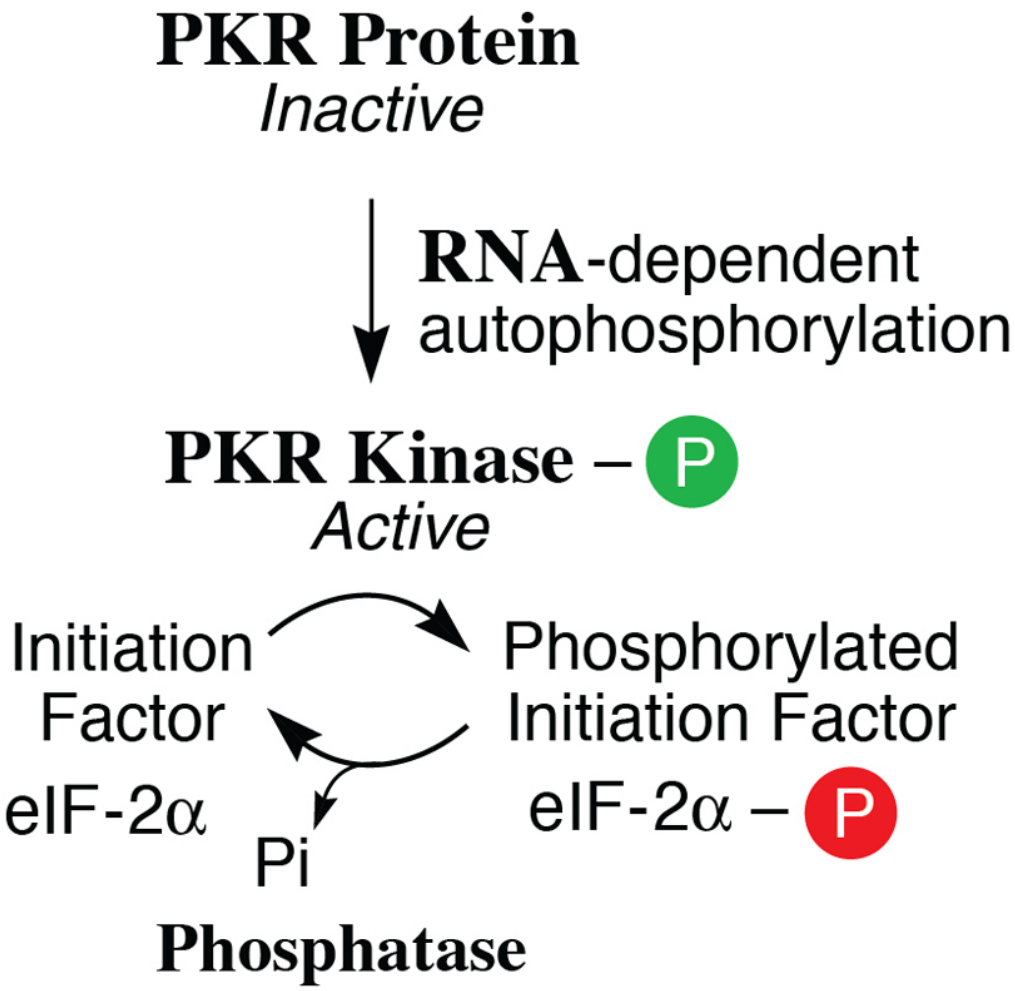


Figure 2. Domain organization of ADAR1 and PKR proteins from human cells and the enzymatic reactions catalyzed by them

(A) Domains. Alternative promoters and alternative splicing give rise to two size isoforms of ADAR1, an IFN-inducible p150 protein and a constitutively expressed p110 protein. A single-sized form of PKR is known that is inducible by IFN. The N-terminal regions of ADAR1 and PKR include repeated nucleic acid binding domains and the C-terminal regions the catalytic domains responsible for their enzymatic activities. Multiple dsRNA binding domains (R_I, R_{II}, R_{III}), three present in both ADAR1 p110 and p150 and two in PKR, are shown in red. RNA adenosine deaminase and protein kinase catalytic domains are shown in yellow for ADAR1 and PKR, respectively. The N-terminal region of the p150 form of ADAR1 also possesses two Z-DNA binding domains (Zα and Zβ, and p110 the Zβ copy, as shown in pink. (B) dsRNA is the substrate of ADAR1. ADAR1 p110 and p150 catalyze the C-6 deamination of adenosine (A) to yield inosine (I) in RNA with double-stranded character. (C) dsRNA is an effector of PKR. RNA-dependent activation of eIF2α protein kinase activity is mediated by autophosphorylation (green P) of PKR; phosphorylation of serine 51 (red P) of eIF2α by PKR leads to an inhibition of protein synthesis.

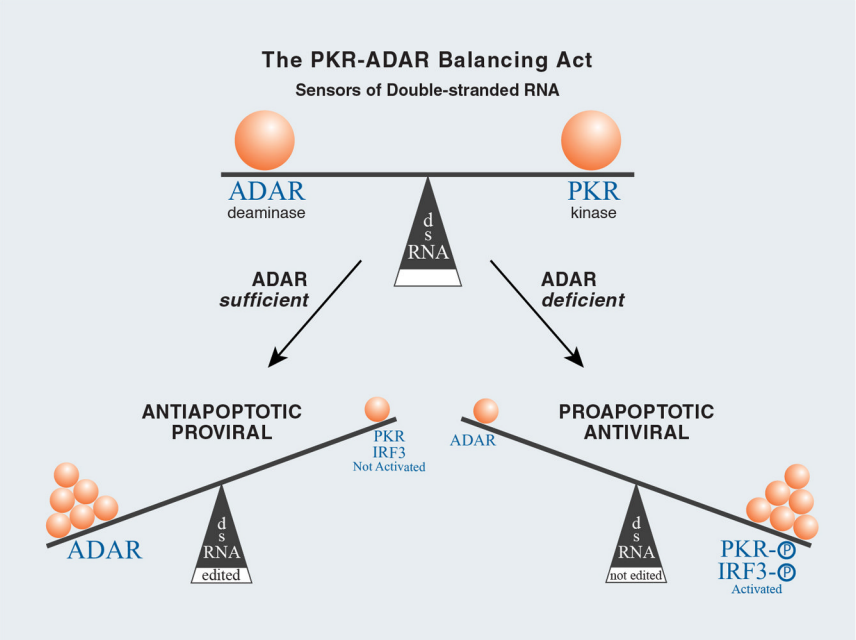


Figure 3. Modulation of innate immune responses mediated by the relative balance between ADAR and PKR acting as RNA sensors

Deamination of adenosine (A) to produce inosine (I) in duplex RNA structures catalyzed by ADAR leads to the nucleotide substitution of an I for an A in RNA. Because I base pairs with C instead of U, dsRNA with I:U mismatch base-pairs are less stable than A:U pairs. ADAR and PKR may also compete for dsRNA. ADAR, and A-to-I editing, may affect gene expression and function in virus-infected cells by a number of mechanisms including suppression of PKR and IRF3 activation.