Tipping the Balance: Antagonism of PKR Kinase and ADAR1 Deaminase Functions by Virus Gene Products

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The protein kinase regulated by RNA (PKR) and the adenosine deaminase acting on RNA (ADAR1) are interferon-inducible enzymes that play important roles in biologic processes including the antiviral actions of interferons, signal transduction, and apoptosis. PKR catalyzes the RNA-dependent phosphorylation of protein synthesis initiation factor eIF-2 α , thereby leading to altered translational patterns in interferon-treated and virus-infected cells. PKR also modulates signal transduction responses, including the induction of interferon. ADAR1 catalyzes the deamination of adenosine (A) to generate inosine (I) in RNAs with double-stranded character. Because I is recognized as G instead of A, A-to-I editing by ADAR1 can lead to genetic recoding and altered RNA structures. The importance of PKR and ADAR1 in innate antiviral immunity is illustrated by a number of viruses that encode either RNA or protein viral gene products that antagonize PKR and ADAR1 enzymatic activity, localization, or stability.

Introduction

INTERFERONS (IFN) WERE DISCOVERED based on their ability to transfer a virus-resistant or antiviral state to previously uninfected cells (Isaacs and Lindenmann 1957; Nagano and Kojima 1958). Interferons exert their actions through signal transduction networks that lead to altered cellular gene expression profiles (Samuel 2001; Sen 2001; Borden and others 2007; Randall and Goodbourn 2008). Among the IFN-induced proteins that play a central role in mediating the varied physiologic changes seen in IFN-treated and virus-infected cells and animals are the protein kinase regulated by RNA (PKR) and the adenosine deaminase acting on RNA (ADAR1). Both PKR and ADAR1 are enzymes that share the property of binding double-stranded (ds) or structured RNAs (Toth and others 2006). The fundamental importance of PKR and ADAR1 as components of the host IFN response against viral infection is emphasized by the fact that a variety of DNA and RNA viruses encode gene products that antagonize the activities of PKR and ADAR1 (Samuel 2001; Katze and others 2002; Haller and others 2006; Randall and Goodbourn 2008). This review will focus on the different strategies by which virus-encoded products, either proteins or RNAs, have been demonstrated to affect the functions of PKR and ADAR1 proteins, including impairment of their enzymatic activities or alteration of the subcellular localization or protein stability as summarized in Table 1.

Protein Kinase Regulated by RNA

The protein kinase regulated by RNA (PKR) is encoded by a single-copy gene located on mouse chromosome 17E2 and human chromosome 2p21-22 (Toth and others 2006). Unlike many genes that are IFN-inducible, a significant basal expression of PKR is often seen in cultured cells and animal tissues (Abraham and others 1999; Shtrichman and others 2002; Zhang and Samuel 2007). The expression of the *PKR* gene is driven by a single promoter that includes a 13-bp ISRE element and a novel 15-bp element unique to the mouse and human PKR promoters that is required for basal as well as optimal IFN-inducible transcription (Tanaka and Samuel 1994; Kuhen and others 1998; Das and others 2006). The PKR protein from human cells is 551 amino acids in length; the N-terminal region includes a repeated domain (dsRBD) that confers dsRNA-binding activity, whereas the subdomains that confer kinase catalytic activity are present in the C-terminal half of PKR (Samuel 1993; Taylor and others 2005b). In response to physiologic stimuli including virus infection, catalytically inactive PKR is activated by a dsRNA-mediated dimerization and subsequent autophosphorylation (Toth and others 2006; McKenna and others 2007a; Sadler and Williams 2007). PKR is normally found in the cytoplasm, and the best characterized substrate of the kinase remains the α -subunit of protein synthesis initiation factor 2 (eIF-2 α), which when phosphorylated on serine 51 leads to an inhibition of translation (Samuel 1979,

TABLE 1. ANTAGONISM OF PKR AND ADAR1 FUNCTIONS BY RNA AND DNA VIRUS GENE PRODUCTS

<i>Evasion strategy</i>	<i>Virus</i>	<i>Gene product</i>	<i>Reference^a</i>
PKR			
RNA binding and sequestration	Vaccinia virus	E3L	Chang and others 1992
	Reovirus	$\sigma 3$	Imani and Jacobs 1988
	Influenza virus	NS1	Bergmann and others 2000; Dauber and others 2006
	Rotavirus	NSP3, NSP5?	Langland and others 1994
	Herpes simplex 1	Us11	Poppers and others 2000; Khoo and others 2002
PKR: viral protein interaction	Epstein-Barr virus	SM	Poppers and others 2003
	Ebola virus	VP35	Feng and others 2007
	Hepatitis C virus	NS5A, E2	Gale and others 1998; Taylor and others 1999
	Influenza virus	P58 ^{IPK}	Melville and others 1999; Goodman and others 2007
	Vaccinia virus	E3L	Romano and others 1998; Sharp and others 1998
Inhibitor RNA	Herpes simplex 1	Us11	Cassady and Gross 2002
	Kaposi's sarcoma	vIRF-2	Burysek and Pitha 2001
	Adenovirus	VA	Mathews and Shenk 1991
Pseudosubstrate	Epstein Barr	EBER	Sharp and others 1993
	Hepatitis C virus	IRES	Vyas and others 2003
	Vaccinia virus	K3L	Carroll and others 1993
eIF2- α P phosphatase activation	Swinepox virus	C8L	Kawagishi-Kobayashi and others 2000
	Ranavirus	eIF2a	Essbauer and others 2001
	Herpes simplex 1	$\gamma 34.5/PP1\alpha$	He and others 1997
Inhibition of activator RNA production	Human papilloma	E6/Gadd34/PP1 α	Kazemi and others 2004
	Measles virus	C protein	Toth and others 2009
	Sendai virus	C protein	Takeuchi and others 2008
Alteration of PKR subcellular localization	Simian virus 5	P/V protein	Gainey and others 2008
	Human papilloma	E6 and E7	Hebner and others 2006
	Human CMV	TRS1 and IRS1	Hakki and others 2006
PKR degradation	Mouse CMV	m142, m143	Child and Geballe 2009; Budt and others 2009
	Poliovirus	Cellular caspase?	Black and others 1989, 1993
	Rift Valley FV	NSs	Habjan and others 2009; Ikegami and others 2009
ADAR1			
RNA binding and sequestration	Vaccinia virus	E3L	Liu and others 2001
	Betanodavirus	B2	Fenner and others 2006
Inhibitor RNA	Adenovirus	VA	Lei and others 1998

^aSee text for additional references.

Abbreviations: ADAR1, adenosine deaminase acting on RNA; CMV, cytomegalovirus; FV, fever virus; PKR, protein kinase regulated by RNA.

2001). PKR is implicated as a key effector of a number of biologic processes including IFN-induced antiviral responses and cellular apoptotic death, through the inhibition of protein synthesis under stress conditions including virus infection and by the modulation of signal transduction processes involving NF- κ B-, p38-, and IRF-3-dependent pathways (Sen 2001; Garcia and others 2006; Sadler and Williams 2007; Toth and others 2006, 2009). Viruses have evolved a number of different and elegant strategies to impair PKR function and hence the PKR-dependent components of the innate antiviral response of cells (Samuel 2001; Haller and others 2006; Langland and others 2006).

Mechanisms of antagonism of PKR function

Inhibition of PKR activation by viral RNAs. Among the naturally occurring antagonists of PKR, the adenovirus VAI RNA was the first characterized inhibitor of PKR and the antiviral response (Kitajewski and others 1986; Mathews and Shenk 1991; Samuel 2001). VAI RNA is an adenovirus-encoded polymerase III gene product that is necessary to maintain protein synthesis at late times after infection (Reich and others 1966).

During infection with dl331, an adenovirus deletion mutant that fails to synthesize VAI RNA, both viral and cellular protein synthesis are considerably reduced (Thimmappaya and others 1982). This inhibition of protein synthesis is due in part to the phosphorylation and subsequent inactivation of translation initiation factor eIF-2 α (Schneider and others 1985; Siekierka and others 1985). VAI RNA, which antagonizes eIF-2 α phosphorylation (Kitajewski and others 1986), binds to PKR both *in vitro* and *in vivo*, which subsequently prevents kinase activation by activator dsRNAs produced during infection (Katze and others 1987; Galabru and others 1989). The yield of mutant virus lacking VAI RNA also is reduced compared to wild-type virus (Kitajewski and others 1986; Zhang and Samuel 2007). Somewhat unexpectedly, the stable depletion of PKR from human cells does not rescue the growth of VAI mutant adenovirus, suggesting that either additional targets of VAI RNA or very low residual levels of PKR (<2%) are sufficient to confer the VAI RNA phenotype (Zhang and Samuel 2007).

Epstein-Barr virus (EBV, human herpes virus 4) encodes 2 small RNAs, EBER-1 and EBER-2, that are synthesized in large amounts in latently infected cells. EBER RNAs are

bound by PKR and prevent kinase autoactivation and eIF-2 α phosphorylation. EBER-1, EBER-2, and VAI RNA exhibit mutually competitive binding to native and recombinant PKR protein (Sharp and others 1993). Interaction of VAI or EBER-1 RNA with PKR leading to inhibition of autophosphorylation employs similar surfaces of interaction as activator RNAs that bind to the dsRBDs (McKenna and others 2006). RNAs encoded by the W repeat region of the EBV genome, in contrast to the EBER RNAs, activate PKR and inhibit translation, suggesting that different products of EBV transcription have the ability to either activate or to antagonize PKR (Elia and others 1996).

TAR RNA of HIV-I represents an additional RNA regulator of PKR function. TAR, present as 59-nt stem-loop-bulge-loop structure at the ends of HIV-I mRNAs (Bannwarth and Gatignol 2005), also occurs as a short 58–66-nt RNA in the cytoplasm of infected cells (Gunnery and others 1992; Kessler and Mathews 1992). TAR RNA interacts with both dsRBM-1 and dsRBM-2 of PKR (Spangord and others 2002; Kim and others 2006). In the cytoplasm, a low concentration of TAR activates PKR, whereas high concentrations interfere with PKR dimerization. Inhibition of PKR may play a role in the maintenance of virus replication in infected cells by decreasing HIV-I frameshift efficiency (Gendron and others 2008). The internal ribosome entry site (IRES) RNA of hepatitis C virus genomic RNA also is bound by PKR, and prevents kinase autophosphorylation and activation (Vyas and others 2003).

The difference in RNA structure between antagonists and activators of PKR remains unclear. VAI RNA contains 3 major domains: a terminal stem domain including base-paired 5' and 3' ends, a central domain, and an apical stem domain (Mathews and Shenk 1991). The apical stem is the primary VAI RNA structure that interacts with the dsRBD RNA-binding motifs of PKR; the VAI central domain also contributes to PKR binding and is responsible for its inhibitory activity (Clarke and Mathews 1995; Ma and Mathews 1996; Spangord and others 2002). The structure and stability of these 2 RNA domains are coupled as they unfold in a single cooperative apparent transition (Coventry and Conn 2008). DsRNA binding and inactivation of PKR are non-equivalent (Bevilacqua and others 1998; McKenna and others 2006). A series of structured synthetic RNA aptamers, selected on the basis of their ability to bind dsRBDs, included both activators and antagonists of PKR autophosphorylation and eIF-2 α phosphorylation, with antagonists binding PKR more tightly than the activators (Bevilacqua and others 1998). The VAI and EBER-I RNA inhibitors prevent self-association and autophosphorylation of PKR and remain associated with PKR under activating conditions, whereas activator dsRNAs dissociate due to reduced affinity for the phosphorylated and autoactivated form of PKR (McKenna and others 2007b). The continued association prevents dimerization of PKR molecules necessary for kinase activity. Thus, both VAI and EBER-I RNA effectively inhibit PKR activation by preventing trans-autophosphorylation between 2 PKR molecules (McKenna and others 2007a, 2007b). However, the ability to activate or antagonize PKR likely is not based on RNA-binding affinity alone. VAI RNA and the synthetic activator poly(rI):poly(rC) have comparable K_D values, while the affinity of TAR RNA is ~100-fold lower (McCormack and Samuel 1995).

Inhibition of PKR by viral proteins that bind RNA, interact directly with PKR, or prevent accumulation of PKR-activating

RNA. Viral proteins inhibit the functions of PKR by a variety of mechanisms including sequestration of the effector RNA, direct protein–protein interactions including formation of inactive PKR heterodimers, acting as viral eIF-2 α pseudo-substrates, and preventing the accumulation of RNAs that lead to PKR activation.

Numerous viral protein antagonists of PKR have been identified, many of which are RNA-binding proteins (Toth and others 2006). Among these are the E3L protein of poxviruses (Chang and others 1992) and the $\sigma 3$ protein of reoviruses (Imani and Jacobs 1988), which were the first characterized protein inhibitors of PKR. Two mechanisms have been advanced to account for the antagonism of PKR by the E3L protein. One mechanism involves sequestering of the PKR activator RNA by E3L (Jacobs and Langland 1996; Shors and others 1997). Indeed, the dsRBDs of PKR and E3L are highly homologous (McCormack and others 1992; Langland and Jacobs 2002). The other mechanism involves direct protein–protein interaction between E3L and the substrate-binding region of PKR to form inactive PKR:E3L heterodimer complexes (Romano and others 1998; Sharp and others 1998). Conceivably both mechanisms are operative, sequestration of activator RNA as well as formation of inactive PKR:E3L heterodimers. The host range of E3L deletion mutant vaccinia virus (Δ E3L) is restricted; Δ E3L-infected HeLa cells show pronounced activation of PKR and eIF-2 σ phosphorylation, impaired late viral protein production, and enhanced apoptosis compared to cells infected with wild-type virus (Garcia and others 2002; Langland and Jacobs 2004). Loss of PKR expression in HeLa cells complements the vaccinia virus E3L deletion mutant (Δ E3L) phenotype by restoration of viral protein synthesis, which correlates with increased virus yields and decreased apoptosis (Zhang and Samuel 2008, 2009).

PKR activation requires RNA binding in most instances, although the cellular protein PACT is able to activate mutant PKR unable to bind dsRNA (Samuel 2001; Sen 2001). Sequestration of activator RNA by binding to a viral protein would represent a mechanism to impair PKR activation and function. In addition to reovirus $\sigma 3$ and vaccinia virus E3L, several more viral proteins are known that bind dsRNA or structured RNA and impair PKR activation. These include the NS1 protein of influenza A virus (Bergmann and others 2000; Chien and others 2004; Li and others 2006); the NS1 protein of influenza B virus (Dauber and others 2006); the NSP3 protein of group C rotaviruses (Langland and others 1994; Yue and Shatkin 1997); the rotavirus NSP5 phosphoprotein that interacts with NSP2 (Vende and others 2002); the Ebolavirus VP35 protein (Feng and others 2007); the EBV SM protein (Poppers and others 2003); and the herpes simplex I (HSV-1, HHV1) Us11 protein (Poppers and others 2000; Khoo and others 2002). In addition to functioning as RNA-binding proteins, the EBV SM protein and the HSV-1 Us11 protein also interact directly with PKR. The Us11 protein contains a domain with homology to eIF-2 α and the C-terminal region of Us11 interacts with PKR and antagonizes kinase activation mediated by PACT (Cassady and Gross 2002; Peters and others 2002). Two hepatitis C virus-encoded proteins, the nonstructural NS5A (Gale and others 1998), and the envelope protein E2 (Taylor and others 1999), as well as the Kaposi's sarcoma herpesvirus HHV8-encoded vIRF-2 protein (Burysek and Pitha 2001), repress PKR function through direct interaction with the kinase in a manner that subsequently impairs eIF-2 α phosphorylation. Similar

to the finding that the knockdown of PKR protein in human cells complements the growth of Δ E3L vaccinia virus (Zhang and Samuel 2008), the knockout of the *Pkr* gene in mice complements the growth of Δ NS1 influenza virus that does not replicate in wild-type mice expressing PKR (Bergmann and others 2000). Influenza virus also employs an additional mechanism to modulate PKR function. Following influenza virus infection, the cellular protein p58IPK dissociates from heat shock protein hsp40; p58IPK subsequently associates with PKR, thereby preventing down-regulation of translation mediated by eIF-2 α phosphorylation (Melville and others 1999; Goodman and others 2007).

In addition to the RNA-binding protein E3L, vaccinia virus and most poxviruses encode a second protein antagonist of PKR function, exemplified by K3L in vaccinia and C8L in swinepox, that acts through a fundamentally different mechanism (Essbauer and others 2001; Samuel 2001). K3L and C8L are homologs of the PKR substrate eIF-2 α . The K3L and C8L proteins resemble the N-terminal substrate-targeting OB-fold domain of eIF-2 α and act as pseudosubstrates to block the phosphorylation of eIF-2 α (Carroll and others 1993; Kawagishi-Kobayashi and others 2000; Seo and others 2008). Various iridovirus isolates from fishes and frogs also encode eIF-2 α homologs (Essbauer and others 2001). Mutations in the C-terminal lobe of the human PKR kinase domain confer resistance to the K3L protein and decrease K3L-binding affinity to PKR, suggesting that subtle changes to the PKR kinase domain may greatly impact pseudosubstrate inhibition without affecting phosphorylation of the natural eIF-2 α substrate (Seo and others 2008). Rapid evolution of the PKR protein has been proposed as a mechanism to evade antagonism by poxvirus K3L and C8L proteins (Elde and others 2009; Rothenburg and others 2009). Substitution of positively selected residues in human PKR with residues found in related species alters sensitivity to viral inhibitors from different poxviruses. Furthermore, differences in sensitivity to poxvirus pseudosubstrate inhibitors occurring between mouse and human PKR proteins have been identified, suggesting that species-specific differences in susceptibility to viral inhibitors of PKR may have important implications in the study of human viral agents in nonhuman model systems (Rothenburg and others 2009).

The observation that the depletion of PKR from HeLa cells can complement the Δ E3L mutation and partially restore viral growth (Zhang and Samuel 2008) is consistent with the conclusion that E3L rather than K3L is the dominant antagonist of PKR at least in HeLa cells (Langland and Jacobs 2004). This finding also is consistent with the notion of formation of inactive E3L:PKR heterodimers, or alternatively, that the RNAs sequestered by E3L are selective in structure or cellular localization in order to discriminate among other RNA-dependent proteins of the innate antiviral response in addition to PKR.

Significant amounts of double-stranded RNA, the best characterized activator of PKR (Sadler and Williams 2007; Samuel 1993, 2001; Sen 2001), have been detected in cells infected with positive-strand RNA viruses and DNA viruses, but not in cells infected with negative-strand RNA viruses including paramyxoviruses (Weber and others 2006). However, activation of PKR and eIF-2 α phosphorylation are seen in cells infected with paramyxovirus mutants defective in expression of P/V/C gene products (Gainey and others 2008; Takeuchi and others 2008; Toth and others 2009).

Sendai virus and measles virus mutants deficient in expression of C protein cause high levels of PKR activation and eIF-2 α phosphorylation relative to their respective wild-type viruses (Takeuchi and others 2008; Toth and others 2009), and the P/V proteins of simian virus 5, which does not encode a C protein, are important factors for antagonizing PKR-mediated translation inhibition (Gainey and others 2008). Therefore, the P/V/C gene products of paramyxoviruses, which are well-characterized virulence factors and determinants of host range and viral pathogenesis, also inhibit PKR function. Inhibition of PKR by paramyxovirus P/V/C gene products does not involve sequestration of dsRNA or direct protein-protein interaction with PKR. Rather, the mechanism appears indirect and involves limiting the generation of activator dsRNAs in infected cells (Takeuchi and others 2008; Toth and others 2009).

Inhibition of PKR by viral proteins that affect phosphatase activity. Alteration of phosphatase activity in a manner that affects the steady-state phosphorylation of eIF-2 α provides an additional, albeit indirect, mechanism to impair PKR function. The herpes simplex virus protein γ 34.5, encoded by HSV-1, is a protein phosphatase 1 (PP1) regulatory subunit that mediates dephosphorylation of eIF-2 α . The γ 34.5 protein interacts with PP1 α phosphatase and redirects the activity to mediate dephosphorylation of eIF-2 α P, which subsequently allows for continued viral protein synthesis (He and others 1997; Jing and others 2004; Zhang and others 2008). Replication of HSV-1 depends on γ 34.5 functions that facilitate virus response to interferon and egress in the different stages of productive infection. γ 34.5 mutants are virulent in *Pkr* knockout mice, but not in wild-type mice. The HSV-1 γ 34.5 protein also binds to the mammalian autophagy protein Beclin 1 and inhibits its autophagy function. The autophagic process initiated by PKR and eIF-2 α phosphorylation targets virions for degradation thereby reducing virus load in the cell (Orvedahl and others 2007). Mutant HSV-1 virus lacking the Beclin 1-binding domain of γ 34.5 fails to inhibit autophagy in neurons and demonstrates impaired ability to cause lethal encephalitis in mice. The neurovirulence of this Beclin 1-binding mutant virus is restored in *Pkr* knockout mice.

Human papilloma virus type 18 (HPV-18) employs a mechanism similar to HSV-1 γ 34.5 to rescue infected cells from PKR-mediated translation inhibition and induction of apoptosis. The HPV-18 oncoprotein E6 interacts with the GADD34/PP1 holophosphatase complex to promote eIF-2 α P dephosphorylation (Kazemi and others 2004). Simian virus 40 large T antigen inhibits PKR-mediated translation inhibition at a step downstream of PKR activation, possibly through dephosphorylation of eIF-2 α P, although the mechanism remains unclear (Rajan and others 1995).

Degradation of PKR. PKR degradation following viral infection has been described in poliovirus (PV)-infected HeLa cells (Black and others 1989, 1993) and Rift Valley fever virus (RVFV)-infected MEF cells (Habjan and others 2009; Ikegami and others 2009). Because PKR is an important mediator of translation inhibition and is involved in a range of processes including limiting virus growth and enhancing cell killing, loss of the PKR protein through proteolysis would provide a mechanism to impair host antiviral innate immune responses dependent upon PKR.

The proteolytic cleavage of PKR was first described in PV-infected cells. Although the PV plus-stranded RNA

genome encodes 2 proteases, PR2A and PR3C, the available evidence indicates that neither of these viral proteases directly cleaves the PKR protein. These PV proteases were expressed as active, recombinant enzymes in *Escherichia coli* and incubated with HeLa cell extracts, but PKR degradation was not observed (Black and others 1993). Rather, a cellular protease activity that is dependent on divalent cations and RNA is implicated, but the identity of the protease enzyme remains unknown. PV-mediated cleavage of PKR depends upon the N-terminus of the PKR protein, the region that includes the dsRNA-binding domains. DsRNA binding by PKR possibly causes an RNA-mediated conformational change that facilitates the proteolysis. PKR kinase catalytic activity is not a prerequisite for proteolysis to occur, as the inactive K296R-mutant PKR protein also is degraded in PV-infected cells (Black and others 1993).

In MEF cells infected with RVFV, a Phlebovirus with a negative-stranded RNA genome, the level of PKR decreases dramatically especially at late times after infection (Habjan and others 2009; Ikegami and others 2009). RVFV-induced loss of PKR is dependent upon the nonstructural s protein, NSs. RVFV-mutant virus deficient in NSs protein expression does not induce the PKR degradation seen with the isogenic wild-type parental virus. Proteasome protease inhibitors MG132 and CLBL restore PKR levels in wild-type RVFV-infected cells, but the caspase inhibitor z-VAD-fmk does not (Habjan and others 2009). The RVFV NSs protein antagonizes PKR-mediated phosphorylation of eIF-2 α and the inhibition of virus protein synthesis and growth. If the NSs protein in RVFV is replaced with an NSs protein from another Bunyavirus, either the less pathogenic sandfly fever Sicilian virus or LaCrosse virus, a Phlebovirus and an Orthobunyavirus, respectively, no reduction in PKR occurs (Habjan and others 2009). RVFV thus appears unique among the bunyaviruses in its ability to mediate the degradation in PKR via a NSs protein-dependent mechanism.

Finally, the cleavage of PKR by caspases has been described in Jurkat T and U937 cells under various stress conditions including those induced by staurosporine, tumor necrosis factor- α with cycloheximide, and anti-Fas antibody (Saelens and others 2001). Pretreatment of cells with the caspase inhibitors Z-VAD-fmk or ZDEVD-cmk abrogated the stress-mediated cleavage of PKR. Asp251 is identified as the site of PKR cleavage by caspases 3, 7, and 8. The C-terminal fragment of PKR that contains the kinase catalytic domain is reported to remain active after caspase cleavage. Because PV induces apoptosis via the caspase cascade (Buenz and Howe 2006), it is tempting to speculate that the degradation of PKR first described in PV-infected cells (Black and others 1989, 1993) and also in cells infected with other viruses where cell killing occurs via apoptosis as exemplified by measles virus (Toth and others 2009), likely reflects caspase-mediated cleavage of PKR.

Changes in subcellular relocation of PKR. Translation is a cytoplasmic process (Lodish and others 2008) and PKR is predominantly a cytoplasmic protein (Thomis and others 1992; Jeffrey and others 1995). Hence, PKR seemingly is appropriately localized within the cell to mediate inhibitory effects on protein synthesis through the well-established mechanism of phosphorylation of translation initiation factor eIF-2 α (Toth and others 2006; Sadler and Williams 2007). Therefore, subcellular relocation of PKR to a region either in the cytoplasm spatially separated from the translational machinery

or alternatively relocation to the nucleus would represent a potentially effective mechanism to suppress PKR function. Subcellular relocation of PKR has been described following viral infection (Dubois and Hovanessian 1990; Jimenez-Garcia and others 1993; Hakki and others 2006; Child and Geballe 2009), for example with human cytomegalovirus (HCMV), murine cytomegalovirus (MCMV), human papilloma virus (HPV), and encephalomyocarditis virus (EMCV). While some studies of PKR in virus-infected cells provide a descriptive correlation between PKR redistribution either aggregated around the nuclei (Dubois and Hovanessian 1990) or in the nucleus (Jimenez-Garcia and others 1993), other studies provide evidence for interaction between specific viral gene products and the PKR protein that correlates with the spatial and functional sequestration of PKR to the nucleus during the course of infection (Hakki and others 2006; Child and Geballe 2009). These viral proteins include TRS1 and IRS1 encoded by human CMV (Hakki and others 2006) and pm142 and pm143 encoded by murine CMV (Child and Geballe 2009).

During HCMV and MCMV infections, PKR accumulates in the nucleus. The HCMV TRS1 and IRS1 gene products expressed in HeLa cells cause a similar relocation of PKR to the nucleus and associated reduction of the cytoplasmic level of PKR and also rescue vaccinia virus Δ E3L virus growth (Hakki and others 2006), whereas a HCMV mutant lacking both TRS1 and IRS1 does not replicate due to enhanced activation of PKR (Marshall and others 2009). A similar relocation of PKR to the nucleus and to insoluble cytoplasmic complexes was observed during MCMV infection. The interaction of PKR with MCMV-encoded m142 and m143 proteins is necessary to achieve relocation of PKR (Child and Geballe 2009). Both m142 and m143 interestingly exhibit PKR inhibitory activity and are essential for virus replication (Valchanova and others 2006; Budt and others 2009). The relocation of PKR by m142 and m143 may contribute to the inhibition of the host antiviral response seen in MCMV-infected cells. Confocal microscopy also indicates that the small delta antigen of HDV and PKR colocalize to the nucleolus; phosphorylation of S-HDAg by PKR affects HDV RNA replication (Chen and others 2002).

Normal human foreskin keratinocytes stably expressing the E6 or E7 oncoprotein of HPV 16 or HPV 31 display altered localization of PKR compared to cells not expressing these proteins (Hebner and others 2006). Increased amounts of PKR are seen in the nuclei of E6-positive normal keratinocytes compared to control cells where PKR is primarily found in the cytoplasm. A similar nuclear localization of PKR is observed with biopsy samples of HPV-positive cervical intraepithelial neoplasia tissue, whereas normal cervical tissue typically does not show nuclear PKR. In the presence of HPV E6, the PKR protein also colocalizes with P bodies, sites of mRNA storage and degradation within the cytoplasm. The relocation of PKR is coupled with reduced levels of both inactive and phosphorylated PKR (Hebner and others 2006).

Redistribution of PKR to the nucleus during viral infection may represent a strategy for viral antagonism of host antiviral defense by redirecting the PKR protein that is a key component of the host innate immune response (Samuel 2001). However, it is conceivable that the relocation of PKR into the nucleus may function as a trigger to activate host defense responses that operate by mechanisms other than

translational inhibition. For example, it is reported that elevated PKR in the nucleus correlates with ER stress-induced apoptosis. Nuclear accumulation of PKR is also described in Alzheimer's disease (Onuki and others 2004), suggesting that redistribution of PKR into nucleus in response to viral infection may indicate physiological or antiviral roles of PKR other than translational control through cytoplasmic phosphorylation of eIF-2 α (Samuel 2001; Toth and others 2006).

ADAR1 Adenosine Deaminase Acting on RNA

The adenosine deaminase acting on RNA (ADAR1) catalyzes the C-6 deamination of adenosine (A) to yield inosine (I) in RNA with double-stranded character (Patterson and Samuel 1995; Samuel 2003). Because I is recognized as G instead of A, such A-to-I RNA editing of viral or cellular RNAs can lead to alterations in coding capacity and changes in RNA structure (Bass 2002; Samuel 2003; Toth and others 2006). ADAR1, like PKR, is encoded by a single-copy gene in mammals. The *ADAR1* gene is located on mouse chromosome 3F2 and human chromosome 1q21 (Toth and others 2006). The expression is driven by 3 alternative promoters, 1 of which is IFN-inducible, that together with alternative exon 1 splicing, give rise to 2 classes of transcripts (George and Samuel 1999; George and others 2005, 2008). In human cells, the IFN-inducible exon 1A containing RNA encodes the long or 1,220 amino acid p150 size form of ADAR1 that is found in both the cytoplasm and nucleus; the constitutively expressed exon 1B and exon 1C containing transcripts encode the short or 931 amino acid p110 size form of ADAR1 that is found predominantly if not exclusively in the nucleus (Patterson and Samuel 1995; Toth and others 2006). Both the p150 and p110 ADAR1 proteins possess a C-terminal adenosine deaminase catalytic domain as well as 3 centrally located copies of the dsRNA-binding domain, similar in sequence to the prototype first discovered in PKR. The IFN-inducible p150 protein is N-terminally extended by 295 amino acids from that of p110, and includes within the N-terminal extension region 2 copies of a Z-DNA-binding motif, the function of which has not yet been clearly defined (Liu and Samuel 1996; Patterson and Samuel 1995; Liu and others 2000; Athanasiadis and others 2005). Both size forms, the p150 and p110 ADAR1 proteins, are active deaminases (Liu and others 1997).

Mechanisms of antagonism of ADAR1 function

In contrast to the multiple strategies by which viruses antagonize the functions of the PKR kinase, comparatively little is known about the antagonism of ADAR1 functions by viral gene products. Two viral antagonists of PKR (Samuel 2001; Garcia and others 2006; Haller and others 2006; Toth and others 2006; Sadler and Williams 2007), the adenovirus VAI RNA (Lei and others 1998; Taylor and others 2005a) and the poxvirus E3L protein (Liu and others 2001), also are known to impair ADAR1 deaminase activity as does the fish betanodavirus B2 protein (Fenner and others 2006).

ADAR1 deaminase activity. The A-to-I editing of viral and cellular RNAs by ADAR1 can be highly selective, occurring at one or a few sites as exemplified by hepatitis delta virus antigenome RNA (Jayan and Casey 2002), herpes virus 8 kaposin K12 RNA transcripts (Gandy and others 2007), human immunodeficiency env RNA transcripts

(Phuphuakrat and others 2008), and the cellular mRNA transcripts for the neurotransmitter receptors for l-glutamate and serotonin (Higuchi and others 1993; Liu and others 1999; Liu and Samuel 1999). These editing events generate protein products with demonstrated physiologic importance and altered function, the result of highly selective amino acid substitutions introduced during translation when I is decoded as G instead of A (Toth and others 2006). In the cases of HDV, HHV8, and HIV, the editing events are proviral. For example, in the case of HDV, large delta antigen is produced by conversion of a stop codon to tryptophan by editing (Casey 2006); in the case of HHV8, the editing levels of the kaposin transcript are nearly 10-fold higher in cells under conditions of lytic viral replication (Gandy and others 2007); and in the case of HIV-1, overexpression of ADAR1 up-regulates HIV-1 virus production and causes editing at a specific site in the env gene, whereas knockdown of ADAR1 by RNAi inhibits HIV-1 production (Phuphuakrat and others 2008). Editing by ADAR1 of viral RNA genomes can also occur at multiple sites during lytic and persistent infections leading to biased hypermutations of the viral RNAs as exemplified by sequence changes described for measles virus (Cattaneo and others 1988), hepatitis C virus (Taylor and others 2005a), and lymphocytic choriomeningitis virus (Zahn and others 2007) RNAs.

The VAI RNA of adenovirus inhibits ADAR1 adenosine deaminase enzymatic activity (Lei and others 1998). VAI RNA also antagonizes A-to-I editing of synthetic dsRNA substrates when tested in extracts from IFN-treated cells or from COS cells expressing either the p110 or p150 size isoforms of recombinant ADAR1. VAI RNA was originally identified as an antagonist of the PKR kinase (Mathews and Shenk 1991; Toth and others 2006). ADAR1 possesses 3 copies of the dsRNA-binding motif that are similar to the 2 copies of the dsRNA-binding motif found in PKR (Toth and others 2006). Analysis of mutants of VAI reveals that the interactions of VAI RNA with ADAR1 and PKR are not equivalent; mutants that do not significantly inhibit PKR autophosphorylation are still able to inhibit ADAR1 deaminase activity (Lei and others 1998). The observation that the growth of mutant adenovirus deleted of VAI is not enhanced in human cells in which PKR is stably knocked down to <5% of control cells indicates the importance of host factors in addition to PKR in conferring the VAI RNA phenotype (Zhang and Samuel 2007). Conceivably, ADAR1 is one of the additional host factors, a possibility that ongoing studies should resolve.

The E3L protein of vaccinia virus also inhibits ADAR1 adenosine deaminase activity *in vitro* (Liu and others 2001). E3L, like ADAR1 p150, contains Z-DNA-binding and dsRNA-binding motifs (Patterson and Samuel 1995; Toth and others 2006). Wild-type E3L protein is a potent inhibitor of deaminase activity measured with a synthetic dsRNA substrate, and mutational analysis revealed that the C-terminal region of E3L containing the dsRNA-binding domain is essential for the antagonism. However, substitution mutations within the Z-DNA-binding domain also abolish E3L antagonism activity. E3L also weakly inhibited the selective editing of glutamate receptor and serotonin 2C receptor pre-mRNA substrates (Liu and others 2001). The B2 protein of fish betanodavirus, like the E3L protein of vaccinia virus, inhibits ADAR1 editing *in vitro* of long dsRNA but is less effective in antagonizing the selective editing of serotonin 2C receptor RNA (Fenner and others 2006).

It has not yet been demonstrated that a viral gene product produced during infection leads to altered A-to-I RNA editing of a viral or cellular substrate by direct antagonism of ADAR1 catalytic activity. However, expression of adenovirus VAI RNA impairs A-to-I editing of HCV replicon RNA in Huh cells and confers IFN resistance to the replicon, and siRNA knockdown of ADAR1 stimulates replicon expression about 40-fold (Taylor and others 2005a). These results taken together suggest that ADAR1 has a role in limiting replication of HCV RNA and that antagonism of ADAR1 by a viral product stimulates viral RNA replication. Whether such antagonism of ADAR1 occurs under conditions of HCV infection by an HCV-encoded gene product is unknown, but is an intriguing possibility, for example, in the case of clinical HCV infections that are resistant to IFN therapy.

Subcellular relocation and degradation of ADAR. The short form of ADAR1, the constitutively expressed p110 ADAR1 protein, is predominately if not exclusively localized to the nucleus, whereas the IFN-inducible p150 or long form is found in both the cytoplasm and nucleus (Patterson and Samuel 1995; Eckmann and others 2001; Poulsen and others 2001; Strehblow and others 2002). While insight has been gained with regard to the subcellular localization of the p110 and p150 size isoforms of the ADAR1 proteins, and the signals responsible for their trafficking (Nie and others 2004; Toth and others 2006), no evidence has yet been presented with regard to virus-mediated subcellular redistribution of ADAR1 in either untreated or IFN-treated cells. Likewise, there are no published reports of ADAR degradation as a result of viral infection similar to what is seen for PKR. However, in principle it is not unreasonable to anticipate that a protein such as ADAR1 that is implicated as an apoptotic factor (Hartner and others 2004, 2009; Wang and others 2004) might be targeted for a caspase-mediated degradation as is described for PKR (Saelens and others 2001).

Conclusions

Considerable progress has been made toward elucidation of the functions of 2 key interferon-inducible proteins, the RNA-dependent protein kinase PKR and the RNA editing enzyme ADAR1, and the strategies by which virus-encoded gene products modulate their activities. The virulence of a virus and the ensuing degree of pathogenesis seen in infections is determined by multiple factors, including activities of PKR and ADAR1. The balance between the actions of PKR and ADAR1 together with other cellular proteins that collectively constitute the innate interferon antiviral response, and the counteractions of viral gene products that have the capacity to antagonize the normal functions of PKR and ADAR1, define the robustness of the interferon-induced antiviral state.

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