INNATE ANTIVIRAL IMMUNITY
REGULATION OF GENE EXPRESSION THROUGH DOUBLE-STRANDED RNA

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Interferon (IFN), the first cytokine discovered approximately 50-years ago during studies on virus interference, was named IFN because of its characteristic ability to interfere with virus growth. The production of type I or viral interferons (IFN-α/β) is an important component of the host innate immune response to infection. Nucleic acid sensors of viral infection that lead to induction of IFN include the cytosolic family of RIG-like receptors (RLRs), the membrane associated Toll-like receptors (TLRs) 3, 7, 8 and 9, and the protein kinase PKR. Recognition of foreign nucleic acids by these sensors is dependent upon the form, structure, size and chemical nature of the 5'-end. Interferons signal through distinct receptors and the canonical JAK-STAT signal transduction pathway to transcriptionally activate the expression of cellular genes. The proteins encoded by these IFN-stimulated genes (ISGs) are responsible for the biologic properties of IFNs. A schematic summary of the IFN system is shown in Figure 1. Among the IFN-regulated proteins (IRPs) are PKR and ADAR1. PKR is a protein kinase regulated by double-stranded (dsRNA) RNA; ADAR1 is an adenosine deaminase that acts on dsRNA. These proteins play important roles in a range of biologic processes including the antiviral actions of interferons (IFN), signal transduction responses, and apoptosis.

The PKR kinase is activated through binding to RNA with double-stranded character. Activation leads to autophosphorylation of PKR, dimerization and subsequent phosphorylation of substrate proteins, the best characterized of which is the protein synthesis initiation factor eIF-2 alpha. Phosphorylation of eIF-2α on serine 51 leads to altered translational patterns in IFN-treated and virus-infected cells. PKR also positively affects signal transduction, including the induction of IFN by dsRNA. The ADAR1 deaminase catalyzes the deamination of adenosine (A) to generate inosine (I) in dsRNAs. Because I is recognized as G instead of A during translation and replication, A-to-I editing can lead to genetic recoding and altered RNA structure. ADAR1 and PKR are both interferon inducible. A single promoter drives the expression of the *PKR* gene, whereas multiple promoters one of which is IFN-inducible drive the expression of the *ADAR1* gene.

The PKR kinase includes in the N-terminal region two copies of a dsRNA-binding domain (RI, RII) that confers the nucleic acid sensing activity, and within the C-terminal region the kinase catalytic subdomains. The IFN inducible form of the ADAR1 deaminase possesses three copies of the conserved dsRNA-binding domain (R1, R2, R3), two copies of the Z-DNA binding domain (Zα, Zβ), and within the C-terminal region the deaminase catalytic domain. The constitutively expressed form of ADAR1 lacks Zα. Schematic diagrams of the human ADAR1 and PKR proteins and their functional domains are shown in Figure 2. The nucleic acid-binding and catalytic activities of PKR and ADAR1 have been established by mutational and biochemical analyses, and their roles in the replication of some animal viruses assessed using cells deficient in the proteins by siRNA knockdown or genetic

knockout strategies or by overexpression strategies.

A single size form of PKR occurs that is predominantly if not exclusively a cytoplasmic protein. Stable clones of human cells deficient in the PKR protein established by an RNA interference silencing strategy show decreased dsRNA-induced apoptosis compared to PKR-sufficient cells. The effect of PKR deficiency on the antiviral response is virus-type dependent. For example, PKR plays an obligatory and major role in restricting growth in human cells of vaccinia virus lacking E3L (ΔE3L). Growth of wild-type vaccinia virus is comparable in PKR-sufficient and -deficient cells, whereas the yield of ΔE3L mutant virus is increased nearly 2 $\log_{10}$ in PKR-deficient compared to PKR-sufficient cells. The increased phosphorylation of eIF-2α seen in ΔE3L-infected PKR-sufficient cells is abolished in PKR deficient cells, and late viral protein production is restored in PKR-deficient cells. PKR also functions as a nucleic acid recognition receptor capable of activating signal transduction pathways. In addition to IPS-1 and IRF3, PKR is a key mediator of type I IFN induction and the induction of apoptosis.

Two-sized forms of the ADAR1 protein are expressed in cultured cells and animal tissues: an IFN inducible ~150-kDa cytoplasmic and nuclear protein and a constitutively expressed N-terminally truncated ~110-kDa nuclear protein. Alternative promoters, one IFN-inducible but STAT1-independent, drive expression of transcripts with alternative exon 1-structures that encode the large- and small-sized ADAR1 enzymes. Mice infected with either microbial or viral pathogens show tissue selectivity of ADAR1 transcript expression, and differential expression in uninfected and infected animals. ADAR1 edits both synthetic dsRNAs and naturally occurring structured RNAs. Among the best characterized substrates of ADAR are the pre-mRNAs for the glutamate-gated ion channel and the serotonin receptor, as exemplified by GluR-B and 5-HT2cR. Altering the information transfer process at the post-transcriptional level of gene expression by A-to-I nucleotide substitution editing through adenosine deamination represents an important strategy for amplifying genetic diversity and modifying protein function. A-to-I editing by ADARs may alter biochemical processes including mRNA translation by changing codons and hence coding potential, pre-mRNA splicing patterns by changing splice site recognition sequences, and viral RNA genomes by changing template and hence product sequences during RNA replication.

The importance of ADAR and PKR is further illustrated by the finding that viruses encode protein and RNA gene products that antagonize their activities. Such antagonism is illustrated by the vaccinia virus E3L protein and the adenovirus VA1 RNA, both of which impair ADAR and PKR function. (Supported in part by NIAID, NIH).

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