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VIRUSES AND INTERFERONS

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■ **Abstract** The interferon system is the first line of defense against viral infection in mammals. This system is designed to block the spread of virus infection in the body, sometimes at the expense of accelerating the death of the infected cells. As expected of potent cytokines, in addition to their antiviral effects, interferons have profound effects on many aspects of cell physiology. All these actions of interferons are mediated by hundreds of interferon-induced proteins that are usually not synthesized in resting cells. Interferons induce their synthesis by activating the Jak-STAT pathways, a paradigm of cell signaling used by many cytokines and growth factors. Surprisingly, some of the same genes can also be induced directly by viruses and double-stranded RNA, a common viral by-product. Some of the interferon-induced proteins have novel biochemical properties and some are inactive as such but can be activated by double-stranded RNA produced during virus infection. Finally, almost all viruses have evolved mechanisms to evade the interferon system by partially blocking interferon synthesis or interferon action. Thus, in nature interferons and viruses maintain an equilibrium that allows regulated viral replication.

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INTRODUCTION TO THE INTERFERON SYSTEM

Interferons (IFNs) are the first known members of the important biological regulatory proteins called cytokines. IFNs were discovered as proteins that inhibit virus replication. They are induced in mammalian cells in response to virus infection, secreted to circulation, and act on as yet uninfected cells to activate a global antiviral state. Thus, IFNs are the mediators of cellular homeostatic responses to virus infection: They are produced in response to viruses and they inhibit virus replication, thereby creating a negative feedback loop. It has become apparent, however, that IFNs do much more than inhibiting virus replication. Like many other hormones, IFNs have pleiotropic effects on many aspects of cell physiology, including cell growth, cell motility, and cell functions. Investigation of the biochemical mechanisms responsible for IFNs' effects has led to the discovery of hundreds of IFN-stimulated genes. How IFNs activate transcription of these genes by transmitting signals from cell-surface receptors to specific genes in the nucleus has been a major question in the field. Relevant studies led to the discovery of the Jak-STAT pathway of signal transduction, a paradigm later shown to be true for many cytokines and growth factors. Although the biochemical functions of most IFN-induced proteins remain unknown, some of the proteins that have been analyzed carry out interesting functions. Moreover, two such proteins use double-stranded (ds) RNA as a cofactor for their enzymatic activity, providing the first biochemical function of dsRNA.

In the context of host-virus infection, regulation of components of the IFN system is multifaceted and complex (Figure 1). Viruses infect cells, and viral gene products, most notably dsRNA, induce the synthesis of IFNs that are secreted. They bind to the extracellular domains of specific cell-surface receptors and transcriptionally activate the IFN-stimulated genes (ISG) whose products inhibit various

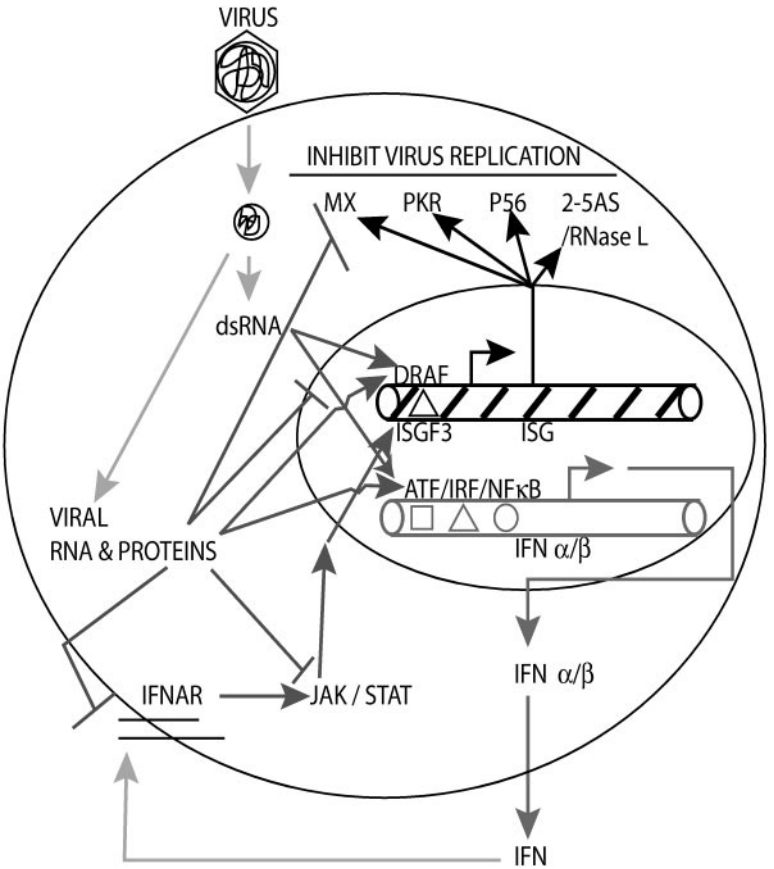

Figure 1 Interactions between viruses and the interferon system. Viruses enter the cell, get uncoated, and synthesize viral RNA and proteins. Often viral dsRNA is also produced. dsRNA can signal to the promoter of interferon (IFN)-α/β genes by activating the transcription factors IRF, NFκB, and ATF. dsRNA can also signal to the promoters of some IFN-stimulated genes by activating the transcription factor DRAF. Induced IFN is secreted from the cells to the culture medium. Secreted IFN binds to the extracellular domain of the cell-surface IFN receptor and activates the Jak/STAT pathway, which leads to the formation of the active transcription factor ISGF3. ISGF3 binds to the promoter regions of IFN-stimulated genes and induces their transcription. Proteins encoded by some ISGs inhibit different stages of virus replication. Viral gene products, other than dsRNA, may also activate IFN genes and ISGs. In contrast, other viral products may interfere with the IFN and dsRNA signaling, binding of IFN to its receptor, and the functions of IFN- and dsRNA-induced proteins.

stages of virus replication. However, some of these antiviral proteins are latent and need to be activated by viral dsRNA. Some ISGs can be directly induced by dsRNA as well, so that the encoded proteins are induced in virus-infected cells without involvement of IFNs. Finally, many viral proteins and RNAs interfere with functioning of the IFN system at every step: IFN signaling, dsRNA signaling, induction of ISGs, and functioning of the induced proteins. Although for the convenience of laboratory investigation many of these processes are studied in isolation, in a virally infected cell or organism they happen simultaneously and affect the overall outcome of the infection process in an integrated fashion. Thus, a dynamic equilibrium is reached between the opposing forces of IFNs and viruses. Experimentally, this equilibrium can be shifted one way by increasing the dose of IFN and the other way by increasing the multiplicity of viral infection.

In this review the molecular aspects of IFN synthesis and IFN action are emphasized from the perspectives of host-virus interactions, whereas other features of the IFN system, such as its role in immune modulation or specific disease therapies, are not described in detail. The reader is referred to several recent reviews that are relevant to the topics discussed here (1, 2, 7, 14, 27, 65, 79, 90, 93). Many original articles, which could not be cited here because of space constraints, are cited in those reviews.

CLASSIFICATION AND PROPERTIES OF INTERFERONS

Interferons are divided into two types, type I and type II, both of which have antiviral activity. The two types of IFNs, however, act through different cell surface receptors and are structurally unrelated. There are many members of the type I or IFN-α/β superfamily but only one member of the type II family, IFN-γ (93). The type I superfamily is divided into four structurally related families: IFN-α, IFN-β, IFN-ω, and IFN-τ. Within the IFN-α family there are 14–20 members, depending on the animal species, but there is only one IFN-β gene in mammals, although 5 such genes exist in ungulates. IFN-τ, or trophoblast IFN, identified in cattle and sheep, is produced in large quantities in the epithelium of the early pre-implantation embryo. The type I IFN genes are all clustered on the short arm of human chromosome 9, and the subspecies of the IFN-α family are 45% homologous in the coding region of the genes, whereas the IFN-β gene is 25–30% homologous to the IFN-α genes. The IFN-α genes and the IFN-β genes are often coordinately regulated because the upstream regulatory regions of these genes have significant homology as well. The cellular source of the different type I IFNs varies: IFN-β, formerly known as fibroblast IFN, is the predominant species produced by various nonhematopoietic cells, whereas IFN-α, formerly known as leukocyte IFN, and IFN-ω are produced by hematopoietic cells.

The type I IFNs are secreted proteins. In all IFN-α proteins, four conserved cysteine residues are thought to form two intramolecular disulfide bonds, one of which is essential for maintaining the biological activity of the protein. Most human IFN-α subspecies are not N-glycosylated, whereas IFN-β is. The tertiary structure

of IFN-β has been analyzed using X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy and is comprised of five α-helices. Both IFN-α and IFN-β function as monomers.

IFN-γ has functional, but no structural, similarity with IFN-α/β. Unlike the intronless type I IFN genes, the IFN-γ gene contains three introns. IFN-γ is a secreted glycoprotein with no cysteine in the mature protein, and the active molecule is a dimer with the N and C termini of the two different subunits constituting the binding site for the receptor. Because there is only limited homology between the IFN-γ species of man and mouse, their action is highly species specific.

REGULATION OF INTERFERON SYNTHESIS

Induction of high levels of type I IFNs is the major initial response to many viral infections of mammalian hosts (7). After this first phase of cytokine response, tumor necrosis factor (TNF), interleukin (IL)-6, IL-12, and IFN-γ are elicited as the second wave of cytokines. At the molecular level, the transcriptional activation of IFN-α/β genes is mediated by complex pathways involving many transcription factors. A well-characterized pathway is triggered by viral double-stranded RNA expressed in infected cells. Other lesser-known pathways that are activated by viral interactions with cellular receptors or by viral proteins must exist as well. The different pathways may differ at the front end, but all converge to the same regulatory regions of the IFN genes to promote their transcription. The IFN-β promoter has been characterized extensively. Several overlapping positive and negative regulatory elements constitute this promoter, and the corresponding proteins, which bind specifically to these elements, regulate transcription (93). In resting cells, in which transcription of the IFN-β gene is off, the elements are occupied by negative factors. They are replaced by positive factors activated in virus-infected cells, causing efficient initiation of IFN-β mRNA transcription. A negative regulatory element acts as a constitutive silencer of the positive NFκB binding site in a distance-independent fashion. A factor, NRF (negative regulatory factor), which mediates this effect (58), interacts directly with NFκB, and a reduction in cellular NRF level derepresses IFN-β gene transcription. A different set of negative regulatory factors is responsible for postinduction shutting off of the promoter: two factors, IRF-2 and PDF1-BF1/Blimp1, bind to the PRDI element, and PRDII-BFI binds to the PRDII element.

Interferon-β Induction

The activation of transcription of the IFN-β gene is driven by the positive elements PRDI, PRDII, and PRDIV, to which the transcription factors IRF-3, NFκB, and ATF-2/c-Jun heterodimer bind, respectively. Cooperative binding of these transcription factors, along with the protein HMGI (y), results in the assembly of the multicomponent enhanceosome complex on the IFN-β promoter. The enhanceosome recruits the coactivator CBP/P300 and the pol II holoenzyme to enhance pre-initiation complex formation (102). The virus-inducible transcription factors

are present in inactive form in the cytoplasm and are activated by phosphorylation. NFκB activation involves activation of the IκB kinase complex and the dsRNA-dependent protein kinase, PKR (protein kinase, RNA-activated) (12). P38 and JNK (Jun N-terminal kinase) kinases are also activated by MEKK1 (microtubule-associated protein, extracellular response kinase kinase) (39). Complete characterization of the activation pathways, however, awaits the identification of additional protein kinases.

A family of transcription factors called IFN regulatory factors (IRF) play important roles in the IFN system (36). Two specific IRF members, IRF-3 and IRF-7, have crucial roles in mediating a cascade of IFN gene induction (53). IRF-3 is expressed widely in all cell types and it is activated by virus infection. Although it is known that the activation is achieved by phosphorylation, the responsible protein kinases and their modes of activation have not yet been elucidated. Activated IRF-3 can bind to the promoters of the IFN-β gene and selected IFN-α genes and induce their transcription. The corresponding IFNs are secreted and, in the second phase of the cascade, they induce the synthesis of IRF-7, which is not expressed constitutively. IRF-7, in turn, induces transcription of other IFN-α genes that cannot be induced by IRF-3. This cascade explains the long-known phenomenon of IFN priming, in which pretreatment of cells with a low dose of IFN boosts IFN production upon virus infection. It also points out that IFN synthesis and IFN action are physiologically intertwined, as demonstrated most convincingly in the IFN-β null mice (17) or the IFN-signaling protein P48 null mice, in both of which virus infection causes poor induction of IFN-α species (79).

Differential Induction of IFN-α Genes

Some members of the IFN-α genes are differentially induced by virus infection, and the underlying mechanisms have been analyzed best in the murine system (66). For example, the IFN-A4 gene but not the IFN-A11 gene is strongly induced in Newcastle disease virus-infected (NDV) cells. As discussed above, one contributing factor is the differential recognition of different IFN-α promoters by IRF-3 and IRF-7. However, additional differential regulation is mediated by the presence or absence of distal negative regulatory elements in the promoters. For the murine IFN-A11 gene, the responsible negative element represses the corresponding positive element in the IFN-A11 promoter only, but does not function as a general repressor. The corresponding binding factor is Ptx1, the pituitary homeobox 1 protein, which is widely expressed in different cell types. The differential effects of Ptx1 were demonstrated by diminishing its expression using antisense technique (51). In wild-type cells, a mixture of IFN-α species is induced, of which, by far, the predominant species is α-4, whereas no α-11 is induced. However, in Ptx-1 nonexpressing cells the spectrum is different: α-5, not α-4, is the predominant species, and α-11 is induced. These results indicate that the regulation of type I IFN gene expression is complex, and it uses several positive and negative pathways to accomplish fine tuning of the repertoire of multiple IFN-α and IFN-β genes.

IFN-γ Induction

IFN-γ is made only by cells of the immune system. Natural killer (NK) cells and T cells are the respective primary producers of IFN-γ during the innate and adaptive phases of immune response to viral infection (7, 93). In general, agents that promote T-cell activation induce IFN-γ synthesis. Infectious agents such as bacteria, protozoa, and possibly viruses trigger IL-12 production by monocytes/macrophages, which in turn act upon NK and T cells to promote IFN-γ synthesis. Nonspecific T-cell activators, such as PHA or ConA, also induce IFN-γ synthesis. Production of IFN-γ is augmented by IL-1, IL-2, growth factors, estrogen, and IFN-γ itself and is inhibited by glucocorticoids, TGF-β, and IL-10. A combination of positive and negative regulatory elements present in the 5′-flanking promoter region and the introns of the IFN-γ gene are thought to govern the cell-specific expression of IFN-γ. However, the details of the IFN-γ induction pathway are not fully elucidated as yet.

RECEPTORS AND SIGNALING

Interferon Receptors

IFN receptors are composed of heterodimers of two type I ectoproteins with single transmembrane domains that do not have intrinsic enzyme activities, but their cytoplasmic domains recruit specific protein kinases that get activated when IFNs bind to the extracellular domains of the receptors (79). The activation process involves receptor dimerization and tyrosine phosphorylation of the receptor proteins as well. The tyrosine kinases involved in this process are members of the Janus kinase (Jaks) family. Activated Jaks phosphorylate themselves, the receptor subunits, and specific members of the family of proteins called signal transducers and activators of transcription (STAT). The STATs are recruited to the receptor-bound Jaks either directly or by SH2 domain-containing adapter proteins that have high affinities for phosphorylated tyrosine residues in proteins. Tyrosine phosphorylated STATs form homo- or heterodimers, translocate to the nucleus, bind to specific DNA sequences on ISGs, interact with the transcriptional machinery and coactivators, and activate transcription. Thus, by physical translocation, the STATs transmit signals from specific receptors on the cell surface to specific genes in the nucleus. The Jak-STAT pathway was first delineated for IFN signaling, and the same paradigm of cell signaling was later found to be operative for many other cytokines and growth factors.

Type I Interferon Signaling

In general, all type I IFNs induce the same set of genes, although there are IFN-β-specific genes as well (68). Type II IFN, in contrast, induces a different but partially overlapping set of genes. The induction characteristics of the type I ISGs

are very similar: Their transcription is fully stimulated within a few hours of cells coming in contact with IFNs. This initial induction does not require ongoing protein synthesis. It is accomplished by IFN-induced activation of STAT1 and STAT2, upon tyrosine phosphorylation by the action of Jak1 and Tyk2 and their assembly with another protein, P48, to form the transcription factor ISGF3, which binds to the specific DNA sequence called IFN-stimulated response element (ISRE) present in the promoter regions of all type I ISGs. Induced transcription of ISGs continues for several hours, during which other ISRE-binding transcription factors, such as IRF-1, probably replace ISGF3 to maintain active transcription. Eventually, the rate of transcription subsides to the basal level through mechanisms that include receptor downregulation, the action of members of the family of proteins called suppressors of cytokine signaling (SOCS) on the Jak tyrosine kinases (21), and the dephosphorylation and degradation of STATs. Thus, gene induction by IFNs is rapid, direct, and transient. The temporary nature of the IFN response is also achieved by the short half-lives of many IFN-induced mRNAs and proteins.

At least seven proteins are essential for type I IFN signaling: the two receptor subunits IFNAR1 and IFNAR2c, the Jak kinases Tyk2 and Jak1, the STAT proteins STAT1 and STAT2, and the DNA-binding protein of the IRF family, P48. Upon binding of IFN to the receptor, the Jak kinases are activated, leading to tyr-phosphorylation of STAT1 and STAT2, which, in complex with P48, form the trimeric transcription factor ISGF3 that binds to the ISRE elements of ISGs in the nucleus and activates transcription. The existence of an additional subtype-specific signaling pathway is indicated by the identification of the βR1 gene, which is preferentially induced by IFN-β (68). IFN-α/β also activates the MAP kinase pathway (16). This could be caused by tyrosine phosphorylation of IFNAR-associated extracellular response kinases (ERKs). Activated ERK can phosphorylate STAT1 on a specific serine residue, which further stimulates the transcriptional activity of that protein. IFN-α-activated STAT1 proteins can also form homodimers that can stimulate transcription of genes containing the gamma-activated sequence (GAS) element.

Type II Interferon Signaling

In contrast to the type I IFNs, IFN-γ induces transcription of genes using multiple mechanisms, some of which need the induced synthesis of new cellular proteins (2). The most well-understood direct response to IFN-γ is mediated by the STAT-1-containing transcription factor GAF (gamma-activated factor), which gets activated by the action of the tyrosine kinases Jak1 and Jak2 and binds to GAS, which is present in the promoter regions of many genes. IFN-γ can also signal to ISRE using a transcription factor distinct from ISGF3, whereas induction of major histocompatibility (MHC) class II genes by IFN-γ is accomplished by an entirely different mechanism involving the transcription factor CIITA (52). A fourth pathway is responsible for inducing the gene encoding P48, a component of ISGF3 and a member of the IRF family (71). Gene induction and transcriptional signaling by both types of IFNs are regulated in a variety of ways. The genes encoding the

Jaks, STATs, and IRFs are often transcriptionally regulated by IFNs and other cytokines, as are the genes for SOCS and protein phosphatases that dephosphorylate STATs. Thus, both positive and negative feedback loops are built into the IFN-signaling systems. Moreover, the signaling pathways used by different cytokines, including IFNs, are often partially overlapping, thus providing the opportunity for cross talks amongst them and causing either synergistic or antagonistic results. Such cross talks are physiologically significant because more than one cytokine is always induced in a virus-infected organism. As a result, cells are often exposed to mixtures of cytokines in vivo, and the resultant patterns of gene expression are dictated by the simultaneous actions of multiple signaling pathways.

The primary IFN-γ signaling pathway leading to the transcriptional activation of genes through GAS elements requires the two subunits of the receptor, IFNGR1 and IFNGR2, Jak1 and Jak2, and STAT1. Tyr-phosphorylated STAT1 forms homodimers, called GAF, which translocate to the nucleus and bind to GAS. In addition, IFN-γ induces Ser phosphorylation of STAT1 by one or more MAP kinases, resulting in a further activated STAT1. IFN-γ can also signal to the ISRE elements using a transcription factor that contains P48. Additional IFN-γ-elicited signaling pathways must exist, because induction of genes encoding the P48 protein or MHC class II proteins is not mediated by GAS or ISRE elements. Similarly, IFN-γ can induce the expression of c-myc and c-jun in the absence of STAT1 (67). Some features of these alternative pathways have been described, but they are not yet fully elucidated.

Constitutive Actions of STAT1

In addition to its role in IFN signaling, STAT1 can participate in constitutive regulation of transcription of genes, a property not dependent on tyr-phosphorylation of the protein (11). Such genes include those encoding caspases 1, 2, and 3, MHC class I, and low molecular mass polypeptide 2 (LMP). The underlying mechanism has been studied in some detail for the LMP2 gene. Unphosphorylated STAT1 and IRF1 bind to each other directly, and the complex binds to two overlapping cis-elements in the gene driving LMP2 gene transcription. STAT1 also represses transcription of several genes (67).

Interferon-Stimulated Gene Induction by dsRNA

Transcription of some, but not all, ISGs can be induced in an IFN-independent fashion by other agents such as dsRNA or virus infection. The responsible pathways are distinct and have been characterized to some degree using cell lines that cannot synthesize type I IFNs or do not respond to them. Such studies have shown that STAT1, STAT2, Jak1, Tyk2, and P48 are not necessary for dsRNA-mediated induction of ISG56, one of the ISGs that is most strongly induced by dsRNA (4, 43). Several transcription factors, named DRAF, VAF, and VIF, have been implicated in dsRNA and virus-mediated induction of ISGs (54, 96, 97, 103). They all contain the transcription factor IRF3 and the coactivator CBP/P300. IRF3

phosphorylation is required for its activation by dsRNA, but the proximal protein kinase has not been identified, although the dsRNA-activated protein kinase, PKR, may be part of the cascade leading to IRF3 activation. A dsRNA-insensitive mutant line has been used to demonstrate that viruses can use yet a third pathway to induce the same genes (30). Although in virally infected cells both IFNs and viral dsRNA can contribute to the induction of ISGs, in some cases mere binding of viruses or viral envelope proteins to the cell-surface receptors can trigger transcription of specific ISGs (8, 56, 108).

INTERFERON-INDUCED PROTEINS

IFNs can induce the synthesis of more than 300 cellular proteins (18). However, the exact pattern of induction varies by cell type and the type of IFN. Moreover, some of the same proteins are also induced by other agents such as dsRNA, LPS, TNF, IL-1, or virus infection. The IFN-induced proteins include enzymes, signaling proteins, chemokines, antigen presentation proteins, transcription factors, heat shock proteins, and apoptotic proteins. The properties of a few of these IFN-induced proteins are outlined below.

2-5(A) Synthetase/RNaseL

2′-5′ oligoadenylate [2-5 (A)] synthetases are a family of enzymes whose synthesis is induced by IFNs (42, 69). There are three structurally related size classes of these enzymes—small, medium, and large—encoded by three separate but clustered genes. Within each class, multiple isozymes with different carboxyl terminal regions are produced by alternative splicing of the primary transcripts. Moreover, different isozymes can have different posttranslational modifications and different subcellular locations. These enzymes polymerize ATP into 2′-5′ linked oligoadenylates of different lengths, but all of them need to be activated by dsRNA using an unknown mechanism. However, a 15–25 bp length of double-stranded region is good enough for activating them maximally (72). Consequently, many viral RNAs with partial ds structures, such as adenoviral VAI RNA, EB viral EBER RNA, and HIV-1 TAR RNA, can activate them (74). The small enzymes are tetramers and the medium isozymes are dimers; their oligomerization is needed for their enzyme activity. The catalytic domain of the P69 isozyme has a structure similar to the catalytic domain of DNA polymerase β with three Asp residues forming the catalytic triad (73).

The 2-5(A) molecules activate the latent ribonuclease, RNase L, by inducing its dimerization upon binding of trimeric or longer 2-5(A) to the protein (77). The 2-5(A) synthetase/RNase L system mediates some of the antiviral and anticellular actions of IFNs. In addition, RNase L has been implicated in specific apoptotic actions (106). Unexpectedly, an RNase-L-independent action of a specific 2-5(A) synthetase isozyme was recently discovered: The 9-2 isozyme causes apoptosis of cells by a process that does not require its enzyme activity but is mediated

by the Bcl2-homology domain 3 present in its unique carboxyl terminal region (25). Through this domain 9-2 can bind to antiapoptotic proteins, such as Bcl2 and BclxL, and block their actions. Thus, 9-2 is a dual-function protein whose synthesis is regulated by IFNs and alternative splicing. Constitutive overexpression of other isozymes of 2-5(A) synthetase can cause an increase in cell doubling time and the formation of multinucleated cells (26). These cellular effects, however, require enzyme activity of the proteins.

PKR

An extensively studied IFN-induced protein is the serine-threonine protein kinase, PKR (13). It is inactive as such and needs to be activated by autophosphorylation. The most well-studied activator of PKR is dsRNA, although other agents, such as heparin, can also activate it. Activated PKR can phosphorylate selected cellular proteins, the most well known of which is the translation initiation factor eIF-2, whose phosphorylation causes an inhibition of protein synthesis. The dsRNA-binding domain of PKR has been studied structurally and functionally. The typical dsRNA-binding motifs present in this domain are shared among many members of this family of dsRNA-binding proteins. The same domain also mediates direct protein-protein interaction, a property that has been exploited to identify PKR-interacting proteins (62). One such protein is P76, a nuclear protein that is a substrate of PKR (63); another PKR-interacting protein is a subunit of a protein phosphatase (101). A third PKR-interacting protein is PACT, an activator of PKR (61). PACT can directly bind to PKR and activate it independently of dsRNA. A small domain of PACT has been identified as the PKR-activation domain, whereas a different domain mediates its strong binding to PKR (64). It has been suggested that PACT may mediate PKR activation in response to cellular stress response (37).

PKR mediates many important cellular functions. In addition to its effects on protein synthesis, PKR is involved in transcriptional signaling in response to IFNs, dsRNA, TNF-α, PDGF, IL-3, and various environmental stresses (99). A common element in many of these responses is the transcription factor NFκB that is activated by PKR. An interesting role of PKR in the splicing of TNF-α mRNA was recently reported (59). PKR has profound effects on cell growth and apoptosis (85). Viral activation of PKR causes apoptosis through a Fas-dependent pathway. Inhibition of PKR action, on the other hand, causes oncogenic transformation of NIH/3T3 cells. These cellular actions of PKR may be mediated by its reported interactions with P53, STAT1, and STAT3. It is clear that PKR is an important mediator of extracellular stimuli that regulate many aspects of cellular physiology. Its pivotal role in host-virus interactions is manifested in the fact that numerous viruses block PKR activation or action using a variety of biochemical strategies.

P56

ISG 56, which encodes P56, is the most strongly induced gene among all ISGs (18). Surprisingly, it is induced very strongly by dsRNA as well, and several

viruses can also induce the same gene independently of the IFN system (30, 89). ISG56 belongs to a clustered ISG gene family, other members of which—P54, P60, and P58—are all structurally related to P56 (95). These proteins contain multiple tetratricopeptide domains that mediate specific protein-protein interactions. The P56 protein is primarily cytoplasmic, and both the protein and the corresponding mRNA are short lived. One cellular function of P56 was revealed by the discovery that it interacts with the P48 subunit of the translation initiation factor, eIF-3 (31). P56 binds to the large multimeric complex of eIF-3 via P48 and blocks its function in initiating protein synthesis (29). P56-mediated inhibition of translation has been demonstrated both in vitro and in vivo, suggesting that the well-known negative effects of IFNs on cell growth may be primarily mediated by the P56 pathway.

Mx and GBP

IFNs efficiently induce the Mx proteins, first identified as the antiinfluenza virus proteins (78). There are both nuclear and cytoplasmic isoforms of Mx, which belong to the dynamin superfamily of GTPases that are involved in endocytosis and vesicle transport. The MxA protein forms large oligomers. A mutant MxA that fails to form oligomers is devoid of GTPase activity. This mutant, however, retains antiviral activity, although it is rapidly degraded in cells (38). In addition to the Mx proteins, another family of guanylate-binding proteins includes GBP-1 and GBP-2. They are also induced by IFNs, have GTPase activity, and could be lipid modified (92).

P200 Family

Members of the P200 family of structurally related proteins are strongly induced by type I IFNs and encoded by a gene cluster (45, 76). The P202 protein impairs cell proliferation by inhibiting the functions of many cellular factors, including NFκB, E2F, P53, c-fos, c-jun, MyoD, myogenin, c-Myc, and Rb (15). The inhibition of c-Myc activity is mediated by blocking its association with Max (94). Another member, P204, is a nucleolar protein that inhibits ribosomal RNA transcription by binding to an essential transcription factor, UBF-1 (50). There are indications that these proteins may have cellular functions beyond the IFN system. P204 is thought to be required for the replication of cytomegalovirus (35). Potential involvements of these proteins in muscle differentiation have been demonstrated. The level of P202 is enhanced during muscle differentiation (15). The level of P204 that is increased during myoblast fusion is due to MyoD-mediated induction of the P204 gene (49). One of the human P200 family members, MNDA, binds to the transcription factor YY1 and stabilizes its binding to DNA (100).

Interferon-Regulatory Factors

The first IFN-regulatory factor, IRF-1, was discovered in the context of IFN synthesis as a transcription factor (57). Other members of this family have since been

identified by virtue of their ability to bind to a consensus DNA element, IRE, and modulate transcription. Because the IRE element closely resembles the IFN-responsive ISRE element, the IRFs affect both IFN synthesis and IFN-mediated gene induction. Expression of several IRF genes is also regulated by IFNs, adding an extra feedback loop. Nine mammalian IRF members, IRF-1 through IRF-9, share significant homology in their DNA-binding domains, which lie at their amino termini. In addition to the cellular IRFs, several viral IRFs have been identified. They are encoded by the human herpes virus 8.

Studies with IRF-1-null mice have shown that this protein plays a prominent role in IFN's antiviral activity. It also participates in cell cycle regulation and apoptosis. Several roles of IRF-1 in the immune system have been identified as well. IRF-2 is a transcriptional repressor, and its overexpression causes oncogenic transformation of NIH/3T3 cells. IRF-3 and IRF-7 are the most important players in the context of viral induction of IFN genes and ISGs (53). They are present in latent forms in the cytoplasm of cells. Virus infection causes their activation by phosphorylation and translocation to the nucleus. IRF-3 is broadly expressed, but IRF-7 is strongly induced by IFNs. These two transcription factors reinforce each other's actions on IFN synthesis. IRF-4 is expressed only in T and B cells and it binds to the transcription factor Pu.1 and activates gene transcription. Consequently, immune cell functions are compromised in IRF-4-null mice. IRF-8 or ICSBP, an IFN-γ-induced protein, is expressed in macrophages and lymphoid cells only. It has an important role in the selection of myeloid cell lineage and macrophage maturation (84). IRF-8-null mice show enhanced susceptibility to virus infection and defects in Th1-type immune response. IRF-9, also known as ISGF3γ or P48, is the DNA-binding component of the transcription factor ISGF3, which mediates type I IFN actions. The viral IRFs interfere with the functions of IRF-3 and IRF-7 and thus block IFN induction. This may be achieved by direct interaction with the transcriptional coactivator, P300, causing an inhibition of its acetyltransferase activity (48).

ANTIVIRAL ACTIONS OF INTERFERONS

Interferons were first recognized and defined by their antiviral properties. Over the years their essential role in mounting the first line of defense against viral infection has been established using tissue cultures and murine model systems. IFN treatment of cells in culture reduces virus yields, and administration of IFNs in vivo reduces viremia and pathogenesis, demonstrating that, experimentally, IFNs can be used as antivirals. That IFNs also play a natural role in regulating the outcome of viral infection was first established by injecting IFN antisera into mice, which exacerbated the infection process. Later, experiments with various knockout mice missing functional IFN genes or genes encoding proteins required for IFN signaling firmly established that without the IFN system the natural viral infection process is more virulent. In vivo the antiviral effects of IFN are mediated both by the immune system and by intracellular antiviral pathways. The latter mechanisms

are responsible for IFNs' direct effects on virus replication and they have been studied primarily in tissue culture.

Unlike the mechanisms of actions of different antibiotics against bacteria, IFNs do not use any magic bullet to block a specific step of viral replication. More than one step of the viral life cycle is usually inhibited by IFNs: Virus penetration and uncoating, transcription of viral mRNAs, synthesis of viral proteins, replication of viral genome, and assembly and release of progeny virions can all be affected by IFNs. For most viruses, IFN impairs more than one step, so there is a cumulative effect on virus yields. The primary targets vary among different virus families and host cell types. The different antiviral pathways are mediated by different IFN-induced proteins. In vivo, experimental elimination of a major antiviral protein is usually compensated for by enhanced actions of others. Moreover, activation of some of the antiviral pathways is dually controlled: They require IFN-induced synthesis of latent enzymes that need to be activated by viral gene products such as dsRNA.

Influenza, Thogoto, and Picorna Viruses

The first connection between a specific virus and a specific IFN-induced protein was made in the case of influenza virus and the Mx family of proteins (78). Genetic analysis of differential susceptibility of different mouse strains to influenza virus led to the discovery of the Mx gene. Different members of this family of proteins reside in the nucleus or the cytoplasm and can inhibit the replication of paramyxoviruses such as influenza virus, Thogoto virus, and rhabdoviruses such as vesicular stomatitis virus (VSV) (32). The antiviral actions against VSV and influenza virus are mediated by different mechanisms because certain Mx mutants can inhibit one but not the other (110). In human MxA-expressing cells, Thogoto viral ribonucleoprotein complexes are retained in the cytoplasm by MxA, thereby preventing its entry into the nucleus (40). Another major connection has been made between the 2-5(A) synthetase/RNaseL system and picornaviruses. Ectopic expression of different 2-5(A) synthetases causes inhibition of replication of encephalo myocarditis virus (EMCV) (26). This effect requires enzymatically active proteins and the presence of RNaseL, indicating the need for 2-5(A) production and activated RNaseL-mediated RNA degradation (33). Viral dsRNA is the likely activator of 2-5(A) synthetases because they coimmunoprecipitate to form an enzymatically active complex (28). Although the RNaseL pathway is the major IFN-induced inhibitory mechanism against picornaviruses, other pathways contribute as well. Consequently, replication of EMCV in mice or cells lacking RNaseL is still inhibited by IFNs, although less severely (107). This is a prime example of multipronged antiviral mechanisms triggered by IFNs to combat replication of even a single family of viruses.

Vesicular Stomatitis Virus

A wide role of the dsRNA-dependent protein kinase, PKR, in impairing virus replication has been suspected because many viruses have evolved mechanisms

to inhibit formation, activation, or action of PKR (22). Activation and action of PKR, as manifested by eIF-2 phosphorylation, have been observed in cells infected with many viruses, suggesting that this enzyme may also play a role in virus-mediated shut-off of host protein synthesis. PKR-null mice and cells have been widely used to assess the contribution of PKR in IFN's effects against specific viruses. Recent investigations (3, 80) show that, in PKR-null fibroblasts, VSV causes massive apoptosis that can be prevented by treating the cells with IFNs. Measurements of virus yield clearly established a major role of PKR in inhibiting VSV replication by blocking viral protein synthesis as well as promoting autocrine IFN production. A dramatic effect of VSV pathogenesis was observed in PKR −/− mice: Intranasal administration of VSV caused massive death of PKR −/−, but not PKR+/+, mice. Surprisingly, neither intravenous nor intraperitoneal injection of VSV caused morbidity. Similar susceptibility of PKR −/− mice was also observed for intranasal administration of influenza virus. These studies clearly demonstrated a critical role of PKR in mediating host response to infection by VSV and influenza virus administered by intranasal routes. In this context, PKR seems to play a dual role by inhibiting viral protein synthesis and promoting apoptosis as well as promoting synthesis of type I IFNs, which have broader effects on virus replication.

Gamma Herpes Virus

Using a similar mouse model of intranasal infection of mouse gamma herpes virus 68, it was demonstrated (20) that, although wild-type mice are very resistant, mice lacking type I IFN receptors are highly susceptible to the virus. To examine the possible roles of two specific IFN-induced proteins in the pathogenesis, similar experiments were done with IRF1-null and iNOS-null mice. Absence of iNOS did not affect the intrinsic resistance of wild-type mice to virus infection, whereas IRF-1−/− mice were highly susceptible. This observation indicates a pivotal role of IRF-1 in host defense against MHV-68, although IRF-1 is not required for resistance against VSV.

VIRAL EVASION OF THE INTERFERON SYSTEM

During the first few decades after the discovery of IFN as the most potent natural antiviral agent, laboratory research was primarily focused on the mechanism of antiviral actions of IFNs. More recently, however, it has become increasingly apparent that almost all families of mammalian viruses are equipped with weapons to protect themselves against the IFN system. Thus, in nature a dynamic equilibrium exists between the IFN system and viruses. In the evolutionary sense, it is in the best interest of the virus to maintain this equilibrium, because a virus, which is a potent inducer of IFN, will propagate poorly if it is also highly susceptible to the host's antiviral actions. Conversely, if the virus multiplies too efficiently and the host is incapable of controlling it, the viral infection will eliminate the host, the reservoir of viruses. For the convenience of investigation, the host-virus

equilibrium is shifted in the laboratory either way by increasing the IFN concentration or the viral multiplicity of infection. At a high IFN dose, viruses are very susceptible, whereas at a high load of virus, IFN is ineffective. Using such experimental maneuvers, it has been possible to demonstrate that a variety of strategies are used by viruses to block different parts of the IFN system. Moreover, most viruses are designed to block more than one step of the IFN system that includes IFN synthesis, its binding to receptors and signaling, induction of ISGs, and functioning of the encoded proteins (Figure 2).

Block in Interferon Synthesis

Several viruses encode proteins that inhibit IFN synthesis. HBV (hepatitis B virus) ORF-C (open-reading frame C) product and the viral terminal protein inhibit IFN-β synthesis (98). The E6 oncoprotein of HPV16 binds to IRF-3 and blocks its ability to activate the IFN-β gene (70). The HPV16 E7 protein binds to IFN-regulating factor 1 through the transactivation domain of IRF-1 (60). As a result, IRF-1-mediated activation of the IFN-β promoter is inhibited. This inhibition was reversed by blocking histone deacetylase activity, suggesting that E7 interferes with the transactivation ability of IRF-1 by recruiting histone deacetylases to the promoter. HHV-8 encodes a viral IRF protein that interferes with the actions of the cellular IRFs and thus blocks IFN induction (109). For influenza A virus, evidence has been presented that NS1 protein blocks IFN induction: A virus lacking this protein is a much better inducer of IFN (83). Overexpression of NS1 affects IFN production by blocking IRF-3 activation in response to not only influenza virus but also Newcastle disease virus (82). Wild-type measles virus is a much poorer inducer of IFNs than the attenuated laboratory strain (55) and it inhibits the induction of IFN by the laboratory strain, indicating that it carries a blocking activity.

Block in Interferon Signaling

Interference with IFN actions starts at the level of receptors. The myxoma virus MT-7 protein can serve as a decoy receptor to bind IFN-γ (91), and vaccinia viral B18R protein can bind IFN-α/β of many species (81). A frequent point of interference by viruses is the Jak-STAT signaling pathway used by IFNs to induce transcription of the antiviral genes. For example, multiple steps of IFN signaling pathways are affected by the adenoviral E1A. It decreases the cellular levels of the signaling proteins STAT1 and P48 and blocks ISGF3 formation (6, 46, 47, 105). In addition, E1A interferes with the interactions between the STAT proteins and CBP/P300 (6, 105). Similarly, E1A also interferes with the constitutive interaction of STAT1 with IRF-1, thereby blocking the constitutive transcriptional activity of STAT1 (10). Other DNA viral gene products known to affect IFN signaling are HBV terminal protein, EBV EBNA1 and EBNA2 proteins, HPV E7 protein, polyoma virus large T, and HCMV and HHV8 proteins (7, 27). Numerous RNA viruses also interfere with IFN signaling. These include influenza virus, ebola virus, Sendai virus, SV5, bovine respiratory syncytial virus (BRSV), parainfluenza virus,

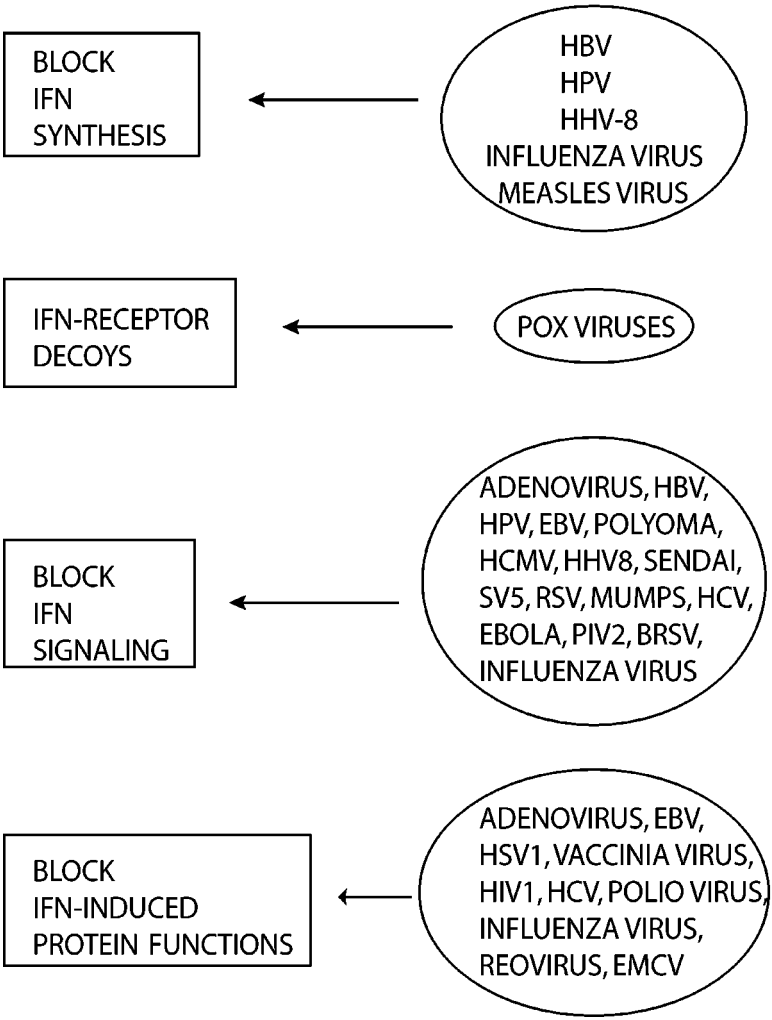

Figure 2 Viral interference with the IFN system. Viruses that can block the four parts of the IFN system are grouped together.

and hepatitis C virus (HCV). Expression of HCV proteins blocked IFN-induced gene expression and the formation of ISGF3 and different STAT dimers (34). The inhibition appears to be at the stage of DNA binding, because STAT tyrosine phosphorylation was not affected. For SV5, the structural protein V has been identified to be the agent for blocking IFN-α/β and IFN-γ signaling. Like adenoviral E1A, SV5 infection caused degradation of STAT1 (19). Sendai virus C proteins interfere with IFN signaling by blocking STAT phosphorylation. In this case, none of the signaling proteins was degraded, and Jak1, but not Tyk2, phosphorylation was

unaffected (41). In the case of BRSV, two nonstructural proteins, NS1 and NS2, cooperated to inhibit both IFN induction and IFN signaling (75).

Block in the Functions of Interferon-Induced Proteins

Another common strategy used by viruses is blocking the functions of selective IFN-induced proteins, which presumably interfere with virus replication. It appears that the most popular target of viral interference is the IFN-induced protein kinase, PKR. This perception could be somewhat misleading because potential effects of viral infection on other IFN-induced proteins have not been seriously examined, primarily because the biochemical functions of most of these proteins are unknown. Among the other IFN-induced proteins of known functions, the 2-5(A) synthetase/RNase L system is known to be affected by herpes virus, SV40, and EMCV (93). Influenza viral NS1 protein has recently been shown to bind to another IFN-induced protein, ISG15, and block its action (104).

Inhibition of PKR's action by various viral gene products exploits a number of different strategies. Several viruses encode RNAs that have partial double-stranded structures, and at high concentrations they block PKR activation by authentic dsRNA. Such viral RNAs include adenoviral VAI RNA, EBV EBER RNA, and HIV-1 TAR RNA (7, 27). Vaccinia virus E3L protein, influenza virus NS1 protein (5), and reovirus sigma 3 protein bind dsRNA and thus can block PKR activation. The vaccinia virus K3L protein and the HIV-1 Tat protein, on the other hand, function as decoys of eIF-2, the natural substrate of PKR, as well (86). Influenza virus activates a novel cellular inhibitor of PKR. This protein, P58, usually stays latent in the cells by being bound to an inhibitory protein. Infection with influenza virus causes the release of P58 and consequent inhibition of PKR. P58 action is thought to be mediated by its binding to a region of PKR that mediates dimerization of the enzyme. The HCV NS5A protein also binds to the same region of PKR and blocks its dimerization, and IFN resistance of different isolates of HCV has been correlated with the ability of the encoded NS5A protein to bind PKR (23). Another protein of HCV, the E2 glycoprotein, has also been shown to inhibit PKR (87). However, the physiological significance of the E2-mediated inhibition remains to be seen. Polio virus and HIV infection cause degradation of PKR through unknown mechanisms. HSV1 encodes two proteins that can interfere with PKR activation or action (86). One of these proteins is Us11, a virally encoded RNA-binding protein (9). The other is the γ_1 34.5 protein. The carboxyl terminus of this protein binds to the cellular protein phosphatase 1α and directs it to dephosphorylate eIF-2α. A recent study confirmed the contribution of the 34.5 protein's action against PKR in HSV-1-mediated pathogenesis. It has been postulated that ICP 34.5 enhances neurovirulence of HSV-1 by blocking the action of PKR. Leib et al (44) showed that a virus lacking the gene encoding ICP 34.5 exhibited attenuated growth and neurovirulence in wild-type mice, but the same mutant virus regained wild-type virulence and growth in mice lacking a functional PKR gene. This is the most convincing study to date that establishes the importance of PKR in viral pathogenesis.

OTHER CELLULAR EFFECTS OF INTERFERONS

Cells of the Immune System

IFNs affect many properties of cells in addition to their ability to support virus replication. The most profound effects are on cells of the immune system that mediate innate and adaptive immune responses (7). Among the different types of IFNs, IFN-γ is the major immunomodulator and primary activator of macrophages that fight off microbial infections. Consequently, mice defective in IFN-γ signaling because of mutations in the IFN-γ, IFN-γ receptor, or STAT1 genes are highly susceptible to infection with Listeria, Toxoplasma, Leishmania, and Mycobacteria (2). The responsible IFN-γ-induced biochemical pathways have been identified in certain cases. Production of reactive oxygen by IFN-γ-induced assembly of NADPH-oxidase is one major pathway; the other is mediated by nitric oxide produced by the IFN-γ-inducible nitric oxide synthase. Using knockout mice, it has been demonstrated that a specific IFN-γ-induced GTP-binding protein, IGT8, is responsible for its action against *Toxoplasma gondii* (88) but not Listeria or cytomegalovirus. IFN-γ has an important role in the development of $CD4^+$ helper T cells as well because it is necessary for directing the differentiation of the $CD4^+$ T cells toward the Th1 direction and away from the Th2 direction. This process is facilitated by enhancing the synthesis of IL-12 in antigen-presenting cells, which in turn drives the development of Th1 cells. In addition, IFN-γ promotes the expression of IL-12 receptors on the surface of $CD4^+$ T cells. Conversely, IFN-γ blocks the production of IL-4, an inducer of Th2 differentiation, as well as the proliferation of Th2 cells. Both type I and type II IFNs promote the expression of MHC class I proteins and the development of $CD8^+$ T cell responses. MHC class II induction occurs in a variety of cell types: mononuclear phagocytes, endothelial cells, epithelial cells, and T cells in which it enhances CD4+ responses. The positive role IFNs play in antigen presentation by inducing MHC proteins is augmented by regulating the expression of many proteins responsible for generating antigenic peptides. A part of this is accomplished by the IFN-γ-mediated induction of three proteasome subunits, LMP2, LMP7, and MECL1. IFN-γ also induces the expression of PA28, the nonenzymatic subunit of proteasome, and TAP1 and TAP2, which transfer the proteasome-mediated peptides from the cytoplasm to the endoplasmic reticulum, where they bind to the nascent MHC class I proteins.

Cell Growth

Another common effect of IFNs is on cell growth and apoptosis. The inhibitory effects on cell growth can be mediated by targeting specific components of the cell cycle control apparatus. The lymphoblastoid EBV-infected Daudi cells are extremely sensitive to the antiproliferative activity of IFN-α. Differential IFN-α sensitivity of different clonal derivatives of Daudi cells has been correlated with the abundance of a viral RNA that has considerable double-stranded structure (24). This RNA may activate PKR, leading to translation inhibition, c-Myc repression,

and NFκB activation, causing cell growth inhibition. IFN-γ may promote cell proliferation or apoptosis depending on the cell type and the presence of secondary signals produced by viral or bacterial infections. Although several proteins are known to suppress the apoptotic activity of IFN-γ in HeLa cells, their modes of action remain to be completely delineated.

Clinical Use

The direct antigrowth property and the immune-simulating properties of IFNs have been exploited successfully in their therapeutic use against several types of cancer (65, 93). Another major disease, multiple sclerosis, is currently managed clinically by administering IFN-β. Among viral diseases, IFN-α/β is clinically used to treat chronic active hepatitis caused by hepatitis C or hepatitis B viruses, and IFN-α is used for treating genital warts caused by papilloma virus infection. Availability of recombinant IFNs and the knowledge of toxicity, pharmacology, route of administration, and side effects of IFNs acquired during the past decade will possibly enhance the future use of IFNs against other diseases.

FUTURE PERSPECTIVES

Although we know much about the IFN system, major gaps in our knowledge remain. For example, the reason for the existence of so many IFN-α subspecies is obscure, although there are suggestions that they may trigger slightly different signaling pathways, leading to different spectra of cellular actions. Similarly, it is clear that IRF proteins play important and multiple roles in the IFN system, but the specific roles played by individual members of this family remain to be deciphered. Regarding the signaling pathways used by IFNs, the major pathway is already well delineated, but the ancillary pathways are ill defined. Our detailed knowledge of the initiation of signaling is not matched by similar understanding of how the signals are downregulated later. In this context, the relative contributions of receptor downregulation, tyrosine phosphatases, SOCS proteins, nuclear proteases, and induction of repressor proteins are unknown. Through cross-talks with other cytokines by sharing STATs, Jaks, SOCS, and tyrosine phosphatases and by crisscross induction of the corresponding genes, IFNs can potentially synergize or antagonize many other cytokines' actions.

It is ironic that we do not know the complete pathway of IFN-mediated inhibition of any virus, most probably because, even for a single virus, IFN uses multiple pathways. It is clear, however, that most viruses have evolved mechanisms to fight the IFN system. The equilibrium attained by these opposing forces is probably optimal for virus propagation in nature because viral mutants that are completely resistant to IFNs do not arise.

A major gap in our knowledge remains in the lack of understanding of the biochemical and cellular functions of most of the IFN-induced proteins. Experimentally, this is a challenging problem, and a major systematic effort will be

required to make meaningful progress. Because many of these same proteins can also be induced directly by virus infection or dsRNA, their cellular functions can be significant, even in the absence of IFNs. Similarly, some of the same proteins are known to be induced in specific tissues at specific developmental stages, indicating major functions in tissue differentiation. Thus, many proteins encoded by the ISGs may have important physiological roles above and beyond the IFN system.

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