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HOW CELLS RESPOND TO INTERFERONS

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ABSTRACT

Interferons play key roles in mediating antiviral and antigrowth responses and in modulating immune response. The main signaling pathways are rapid and direct. They involve tyrosine phosphorylation and activation of signal transducers and activators of transcription factors by Janus tyrosine kinases at the cell membrane, followed by release of signal transducers and activators of transcription and their migration to the nucleus, where they induce the expression of the many gene products that determine the responses. Ancillary pathways are also activated by the interferons, but their effects on cell physiology are less clear. The Janus kinases and signal transducers and activators of transcription, and many of the interferon-induced proteins, play important alternative roles in cells, raising interesting questions as to how the responses to the interferons intersect with more general aspects of cellular physiology and how the specificity of cytokine responses is maintained.

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INTRODUCTION

Type I (predominantly α and β) and type II (γ) interferons (IFNs) signal through distinct but related pathways. Enormous progress has been made in recent years in understanding how cells respond to IFNs, especially in uncovering the pathways that mediate inducible gene expression. We now know that these pathways involve (*a*) specific type I and type II receptors, which bind to the Janus kinases (JAKs), and (*b*) the signal transducers and activators of transcription (STATs), which in turn propagate the signals. Moreover, JAKs and STATs, discovered through investigations of IFN signaling, also are involved in many different cytokine- and growth factor–mediated pathways. We know of four mammalian JAKs and seven STATs. Several recent reviews describe signaling by IFNs in relation to other cytokines and growth factors (1–5) and more general aspects of JAK-STAT function and the family relationships (6–10).

After activation by JAKs through phosphorylation of a specific tyrosine residue, STATs form homo- or heterodimers through mutual phosphotyrosine-Src homology region 2 (SH2) interactions. STAT dimers bind to gamma-activated sequence (GAS) elements, which drive the expression of nearby target genes. Different GAS elements prefer different STAT dimers, helping to establish specificity. Both STAT1-2 heterodimers and STAT1 homodimers bind to p48, a member of the interferon regulatory factor (IRF) family. The resulting trimers—called IFN-stimulated gene factor 3 (ISGF3) in the case of the STAT1-2 heterodimer—bind to IFN-stimulated regulatory elements (ISREs) that are distinct from the GAS elements. ISREs drive the expression of most IFNα/β-regulated genes and a few IFNγ-regulated genes. This review describes the signaling pathways used to turn the IFN responses on and off and the functions of the induced proteins in mediating the major cellular responses to IFN. Many of the proteins involved in both signaling and responses have important alternative functions, which are also reviewed.

SIGNALING PATHWAYS

Interferon γ

The proximal events of IFNγ signaling require the obligatory participation of five distinct proteins: type I integral membrane proteins IFNGR1 and IFNGR2 (the subunits of the IFNγ receptor) and JAK1, JAK2, and STAT1 (2, 11). Recent work has revealed that this signaling pathway is necessary, though not always sufficient, for induction of most if not all IFNγ-dependent biological responses in vitro and in vivo. IFNγ receptors are expressed on nearly all cell types, with the possible exception of mature erythrocytes, and display strict species specificity in their ability to bind IFNγ (12). Functionally active IFNγ receptors consist of at least two species-matched polypeptide chains (Figure 1). IFNGR1 (previously the α chain or CD119w), a 90-kDa polypeptide encoded by genes on human chromosome 6 and murine chromosome 10, plays important roles in mediating ligand binding, ligand trafficking through the cell, and signal transduction (11, 12). IFNGR2 (previously the β chain or accessory factor-1),

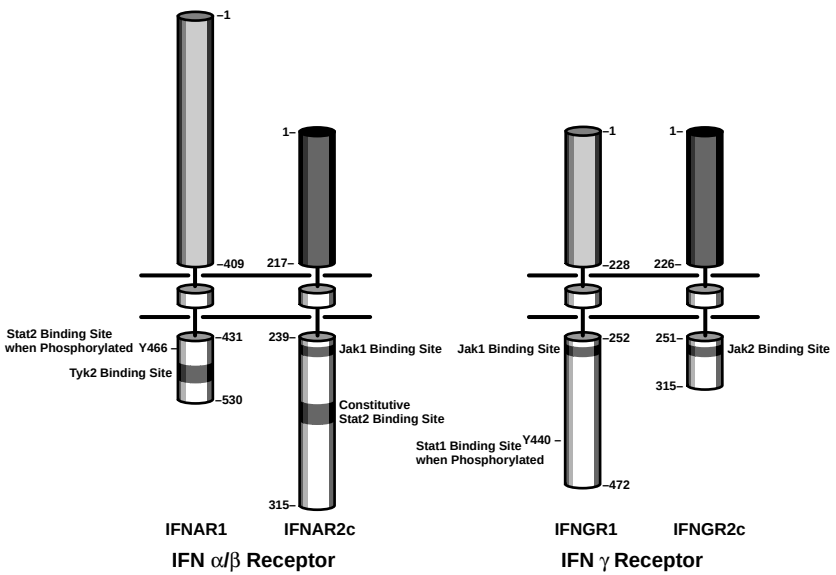


Figure 1 Schematic diagram of the human interferon (IFN) α/β and IFNγ receptors. (*left*) The IFNAR1 and IFNAR2c subunits of the IFNα/β receptor. (*right*) The IFNGR1 and IFNGR2 subunits of the IFNγ receptor. The positions of amino acid residues are shown inside each subunit, and functionally important intracellular domains are also identified. STAT: signal transducer and activator of transcription; JAK: Janus kinase.

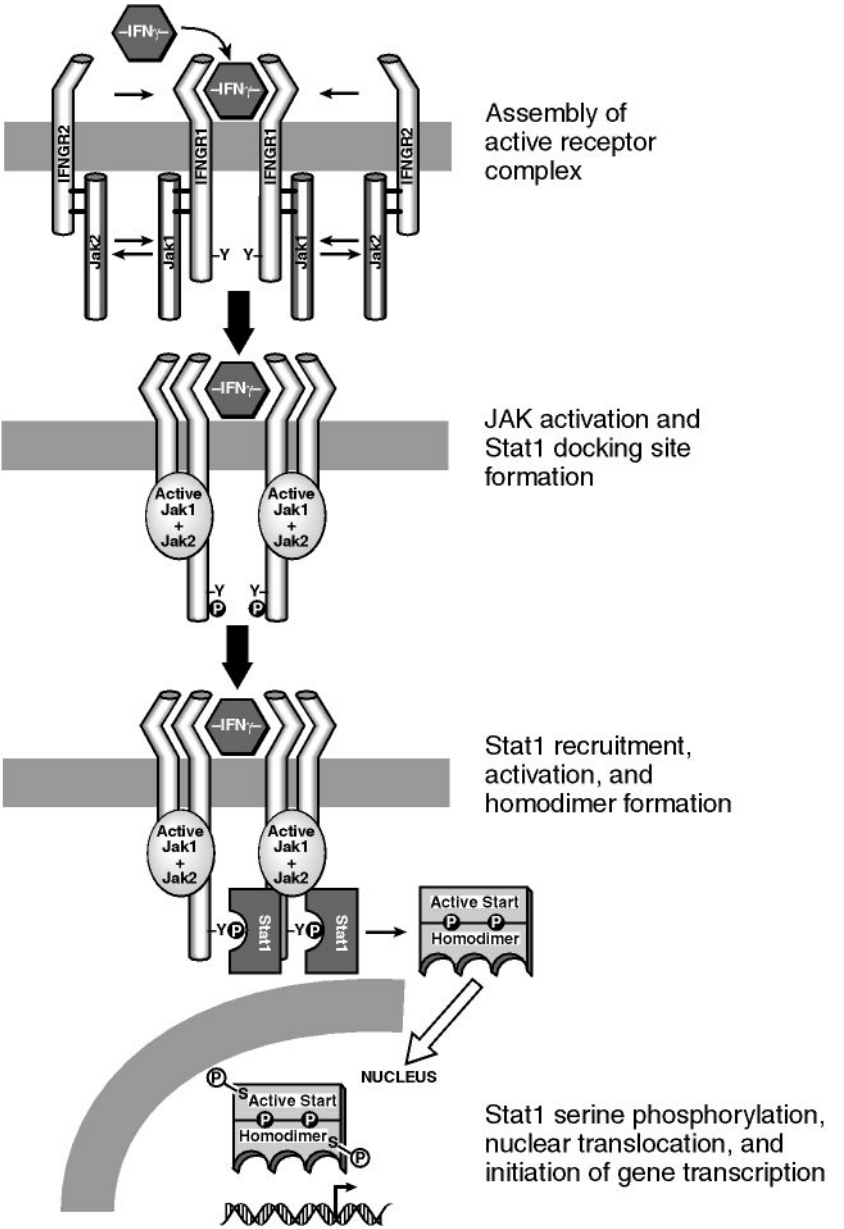
a 62-kDa polypeptide encoded by a gene on human chromosome 21 and murine chromosome 16, plays only a minor role in ligand binding but is required for signaling (11, 13, 14).

Three sets of experiments have implicated JAKs and STATs in mediating IFNγ-dependent cellular responses. First, isolation and complementation of mutant human cell lines have revealed that JAK1 and JAK2 become selectively activated in IFNγ-treated cells and are required for the ligand-dependent activation of IFNγ-inducible target genes (1). Second, through biochemical approaches, STAT1—a novel latent cytosolic transcription factor—was isolated and shown to undergo rapid tyrosine phosphorylation and activation in IFNγ-treated cells (1, 2). Third, structure-function analyses of the intracellular domains of the two IFNγ receptor subunits identified constitutive, specific binding sites for JAK1 and JAK2. Moreover, IFNγ induced the formation of a specific phosphotyrosine binding site on the receptor for STAT1, thereby providing the mechanism linking the activated receptor to its signal transduction apparatus (11).

Based on these and other observations, a relatively complete model of IFNγ signaling has been formulated (Figure 2). In unstimulated cells, the IFNγ receptor subunits do not preassociate with one another strongly (15), but their intracellular domains associate specifically with JAK1 and JAK2 (15–18). JAK1 binds to IFNGR1 through a 4-residue sequence ($_{266}$LPKS$_{269}$) in the membrane-proximal region of the IFNGR1 intracellular domain. JAK2 binds to a 12-residue, proline-rich Box 1–like sequence ($_{263}$PPSIPLQIEEYL$_{274}$) in the membrane-proximal region of the intracellular domain of IFNGR2.

Functionally active IFNγ is a homodimer that binds to two IFNGR1 subunits, thereby generating binding sites for two IFNGR2 subunits (15, 19–22). Within the resulting symmetrical signaling complex, the intracellular domains of the receptor subunits are brought into close proximity, together with the inactive JAKs that they carry. JAK1 and JAK2 are then sequentially activated by auto- and transphosphorylation. Activation of JAK2 occurs first and is needed for the subsequent activation of JAK1, which has a structural as well as enzymatic role (23). Work with chimeric JAK1 proteins and receptors has shown that the

Figure 2 Signaling through the interferon (IFN)γ receptor. The details of this model are described in the text. In unstimulated cells, IFNGR1 associates with Janus kinase (JAK)1, and IFNGR2 associates with JAK2. IFNγ induces oligomerization of the IFNγ receptor subunits, which leads to the transphosphorylation and activation of JAK1 and JAK2. The activated JAKs then phosphorylate Y440 of IFNGR1, creating a docking site for signal transducer and activator of transcription (STAT) 1. While bound to the receptor, STAT1 is phosphorylated on Y701 and is released from the receptor, forming a homodimer that translocates to the nucleus.


IFN-γ
IFNGR2
IFNGR1
IFNGR1
IFNGR2
Jak2
Jak1
Y Y
Jak1
Jak2
Assembly of active receptor complex
Active Jak1 + Jak2
JAK activation and Stat1 docking site formation
Stat1
Active Start
Homodimer
Stat1 recruitment, activation, and homodimer formation
NUCLEUS
Stat1 serine phosphorylation, nuclear translocation, and initiation of gene transcription

specificity of JAKs lies in their capacity to associate with particular cytokine receptor subunits rather than because of a high degree of substrate specificity (24, 25).

Once activated, the receptor-associated JAKs phosphorylate a functionally critical, tyrosine-containing five-residue sequence ($_{440}$YDKPH$_{444}$) near the C terminus of IFNGR1, thereby forming paired ligand-induced docking sites for STAT1 (26–28). Two latent STAT1 proteins then bind to these sites because the SH2 domain of each recognizes the tyrosine-phosphorylated YDKPH sequence (28, 29). The receptor-associated STAT1 proteins are thus phosphorylated by the receptor-bound kinases at tyrosine 701, near the C terminus (30–32). The phosphorylated STAT1 proteins dissociate from the receptor and form a reciprocal homodimer, which translocates to the nucleus by a mechanism dependent on the GTPase activity of Ran/TC4 (33). The active STAT1 homodimers bind to specific GAS elements of IFNγ-inducible genes (1, 2) and stimulate their transcription. The transcriptional activity of STAT1 homodimers is enhanced at some point in the activation cascade by serine phosphorylation (at position 727) by an enzyme with MAP kinase–like specificity (34, 35).

Thus, the biological responses of cells to IFNγ result from ligand-dependent, affinity-driven assembly of a multimolecular signal transduction complex that derives at least some of its specificity from selective recruitment of only one member of the STAT family to its ligand-induced, tyrosine phosphate–docking site on the receptor. Importantly, subsequent work in other labs has shown that other members of the STAT family are recruited to their respective cytokine receptors by similar ligand-induced mechanisms. As a result, the IFNγ signaling model is now an accepted paradigm that explains an important mechanism of how cytokine receptors are coupled to their specific STAT signaling systems.

Processes that negatively regulate IFNγ signaling are only now being defined. In certain cells, such as T cells, IFNγ can induce desensitization by down-regulating the expression of the IFNGR2 mRNA and protein (36, 37). However, whether this mode of desensitization occurs in other cell types remains unclear. Dephosphorylation of the activated IFNGR1 subunit occurs rapidly following stimulation with IFNγ (26–27). However, because no data suggest that the IFNγ receptor associates with a particular phosphatase, dephosphorylation of the receptor may result from the action of general cellular phosphatases. More work in this area is needed. IFNγ (as well as several other cytokines) can induce the expression of a family of proteins termed SOCS/JAB/SSI, which bind to and inhibit activated JAKs (38–40). This work reveals that cytokines can desensitize cells in either a homologous or a heterologous manner by inducing proteins that block JAK activity. Although some of these proteins have been shown to inhibit IFNγ-induced biological responses when overexpressed

in cells, little information is available to define their enzyme or cytokine specificities. Nevertheless, further investigation of those novel proteins is likely to produce new insights into how JAK-STAT pathways are regulated.

The physiological relevance of IFNγ signaling through the JAK-STAT pathway and the basis for signaling specificity has been established unequivocally through the generation and characterization of mice with a targeted disruption of the STAT1 gene (41, 42). STAT1-null mice show normal tissue and organ development, produce normal numbers and distributions of immune cell populations, and are able to reproduce. However, cells from these mice are incapable of manifesting any biologic responses to either IFNγ or IFNα, and the mice display severe defects in the ability to resist microbial and viral infections. In contrast, STAT1-null mice do not display abnormalities in responses induced by a variety of other cytokines [such as growth hormone, epidermal growth factor (EGF), and interleukin (IL)-10] that have been shown to activate STAT3 and STAT1 in vitro. Taken together, these results show that under physiological conditions, the development of biological responses induced by IFNγ (and in many cases also by IFNα/β) requires the participation of STAT1. These results further suggest that the use of STAT1 in signaling pathways under physiological conditions is restricted largely to the IFN systems. Thus, the specificity of IFNγ signaling is due predominantly to two temporally and topographically distinct processes involving STAT1. The first process is the recruitment of STAT1 to a specific docking site formed on the activated receptor at the membrane. In the second process, activated STAT1 dimers, once they arrive in the nucleus, activate a distinct set of cytokine-inducible genes.

The ability of STAT1 to activate gene expression may also be modulated by its interaction with other transcription factors. For example, IFNγ-dependent induction of the 9-27 gene is mediated by the interaction of a STAT1 homodimer-p48 complex with an ISRE rather than with a GAS element (43, 44). In addition, induction of the ICAM-1 gene by IFNγ depends on the interaction of STAT1 and the transcription factor Sp1, which occurs when both proteins are bound to DNA (45). Thus, cell type–specific gene induction by IFNγ may be explained, at least in part, by the ability of additional cell-specific positive and negative factors to modulate the actions of STAT1 (46, 47).

Interferons α and β: The Common Pathways

The main pathway of response to IFNα/β requires two receptor subunits, two JAKs, two STATs, and the IRF-family transcription factor p48 (Figure 3). The IFNα/β signaling pathways are understood at least as well as any other, but we are still at a relatively early stage, capable of drawing blobs to illustrate the major interactions but ignorant of the fine mechanistic detail. It will require analysis of the three-dimensional structures of the major components, individually and

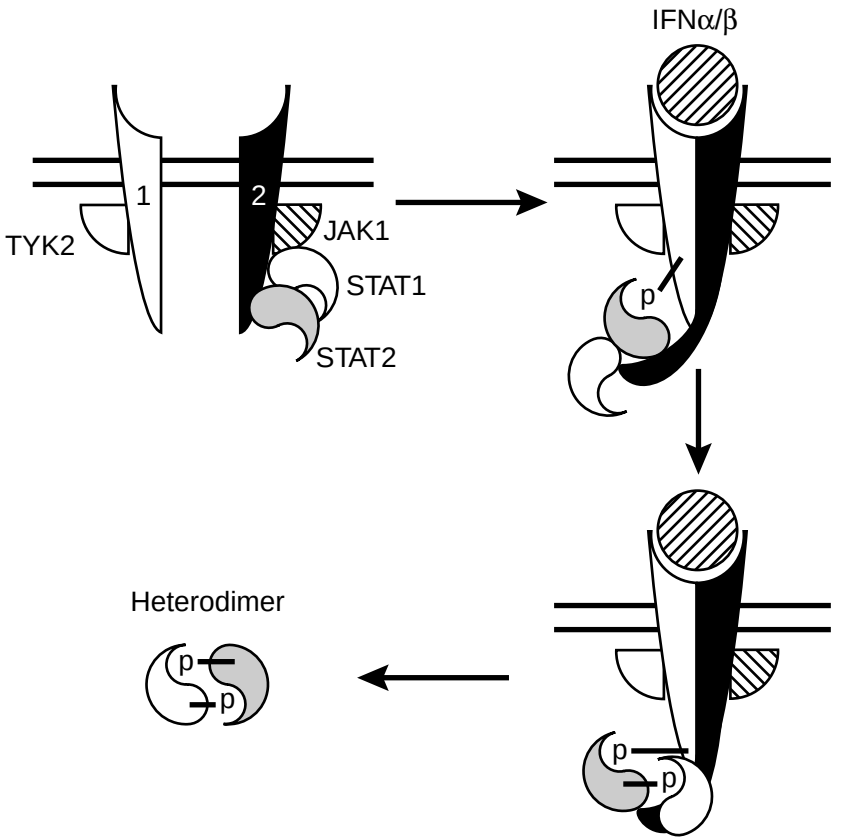


Figure 3 A model for the ordered formation of signal transducer and activator of transcription (STAT) 1 and STAT2 heterodimers at the interferon (IFN)α/β receptor. In the unliganded receptor, IFNAR1 associates with Tyk2, and IFNAR2 associates with Janus kinase (JAK) 1, STAT1, and STAT2. The binding of STAT1 to IFNAR2 depends on STAT2 but not vice versa. The IFN-mediated association of IFNAR1 and -2 facilitates the cross-phosphorylation and activation of Tyk2 and JAK1, which in turn phosphorylate Y466 of IFNAR1, creating a docking site for the SH2 domain of STAT2. This new interaction positions STAT2 for phosphorylation on Y690, thus creating a docking site for the SH2 domain of STAT1, positioning it for phosphorylation on Y701. Release of the STAT1-2 heterodimer from the receptor follows.

complexed with one another, and then manipulation of those structures to reveal the detailed interactions that are crucial for function.

The overall plan of IFNα/β signaling (Figure 3) involves five major steps: (*a*) IFN-driven dimerization of the receptor outside the cell leads to (*b*) initiation of a tyrosine phosphorylation cascade inside the cell, resulting in (*c*) dimerization of the phosphorylated STATs, activating them for (*d*) transport into the nucleus, where they (*e*) bind to specific DNA sequences and stimulate transcription. Current understanding of this initial part of the response is greater than of the full response, which additionally involves the suppression of IFN-stimulated genes (ISGs) in the absence of IFN and down-regulation of the initial response in the continued presence of IFN. In addition to this main pathway, IFNα/β activates several other pathways. Although the biochemical evidence for additional signaling is persuasive, unfortunately we still have little knowledge of the physiological roles. It is also clear that different IFNα/β subtypes can stimulate distinct and different ancillary responses. As discussed below, the mechanism probably involves a novel pathway in addition to the one shown in Figure 3.

The receptor has two major subunits (Figure 1): IFNAR1 (the a subunit in the older literature) and IFNAR2c (the β_L subunit). IFNAR2a is a soluble form of the extracellular domain of the IFNAR2 subunit (48), and IFNAR2b (also called the β_S subunit) is an alternatively spliced variant with a short cytoplasmic domain (48) that, when overexpressed, can have dominant negative activity (49). Only IFNAR2c restores IFNα/β signaling to a mutant cell line in which the IFNAR2 gene has been inactivated (50). In contrast to the situation for the IFNγ receptor, neither IFNAR1 nor IFNAR2 alone binds to IFNα/β with the high affinity of the two-subunit combination (51, 52). After IFNα/β is bound, the cascade begins with the phosphorylation of Tyk2, which is preassociated with IFNAR1 (53, 54). JAK1, bound to IFNAR2c, can phosphorylate and activate Tyk2 (55), which can then cross-phosphorylate JAK1 to activate it further. Tyk2 also plays a structural role because the amount of IFNAR1 is low in Tyk2-null cells (56). The domains required for this role are distinct from those required to transduce the signal (57). Activated JAK1 and Tyk2 are almost certainly responsible for the sequential phosphorylation of Y466 of IFNAR1 (58), Y690 of STAT2, and Y701 of STAT1.

As shown in Figure 3, both STAT1 and STAT2 preassociate with IFNAR2c in untreated cells (59). STAT2 binds in the absence of STAT1, but STAT1 binds well only to the IFNAR2c-STAT2 complex (59). STAT1 and STAT2 also seem to associate with each other in the cytosol of untreated cells (60), but the physiological significance of this interaction is unclear. When Y466 of IFNAR1 is phosphorylated, the SH2 domain of STAT2 binds to it (61), followed by the phosphorylation of both STATs and dissociation of the phosphorylated heterodimer from the receptor.

Experiments in which the SH2 domains of STAT1 and STAT2 have been interchanged reveal that the specificity of the IFNγ receptor for STAT1 requires the SH2 domain (29) because a variant STAT1 carrying the SH2 domain of STAT2 does not function. However, in the case of the IFNα/β receptor, a very different result is obtained. STAT2 works equally well with either its own SH domain or that of STAT1 (29, 59), revealing that different domains of STAT2 are more important in establishing specific interactions with the receptor. The N-terminal third of STAT2 has been identified as the dominant subregion in determining specificity (59).

In addition to the seven major components discussed above, there is evidence that the tyrosine phosphatase SHP-2 may also be required for signaling. This enzyme preassociates with IFNAR1 and is phosphorylated in response to IFNα/β (62). In transient cotransfection experiments, a dominant negative form of SHP-2 inhibits the IFNα/β-induced expression of a reporter gene (62). Experiments showing that SHP-2 is required for IFNα/β-dependent activation of endogenous genes in untransfected cells would help to complete this interesting story.

How activated STATs reach the nuclei of IFN-treated cells is not yet clear. ISGF3, the major transcription factor formed in response to IFNα/β (Figure 4), is required to drive the expression of most ISGs via their ISREs, as shown by the specific defects in p48-null human cells (63, 64). Interestingly, p48-null mice also show severe defects in the induction by viruses of the IFNα/β genes themselves, consistent with the binding of ISGF3 to virus-inducible elements within the IFNα/β promoters (65). STAT1-2 heterodimers and STAT1 homodimers form in response to IFNα/β independently of p48, and each can drive the expression of a minority of ISGs, such as the IRF1 gene, through GAS elements (66, 67). The relative amounts of STAT1-2 heterodimer and ISGF3, or of STAT1 homodimer and its complex with p48 (64), will obviously depend on the levels of p48, which can vary widely among different cell types. Because the STAT1 homodimer that forms in response to IFNα/β does not drive the expression of IFNγ-responsive genes that contain GAS elements, it stands to reason that an additional response to IFNγ is required and that a secondary modification of STAT1 homodimers in response to IFNγ may be involved (66). A prime candidate is the phosphorylation of serine 727 of STAT1 (34).

Initial analysis of the interaction of ISGF3 with the 6-16 and 9-27 ISREs showed that the protected region is about 35 nucleotides long (68, 69). The most meaningful contacts between the ISRE and ISGF3 involve STAT1, with p48 playing a less important role and STAT2 serving to provide a potent transactivation domain (70, 71). The region between residues 400 and 500 of STAT1 provide binding-site specificity (72), and the region between residues 150 and 250 is involved in contacting the C-terminal portion of p48 (73). The STAT

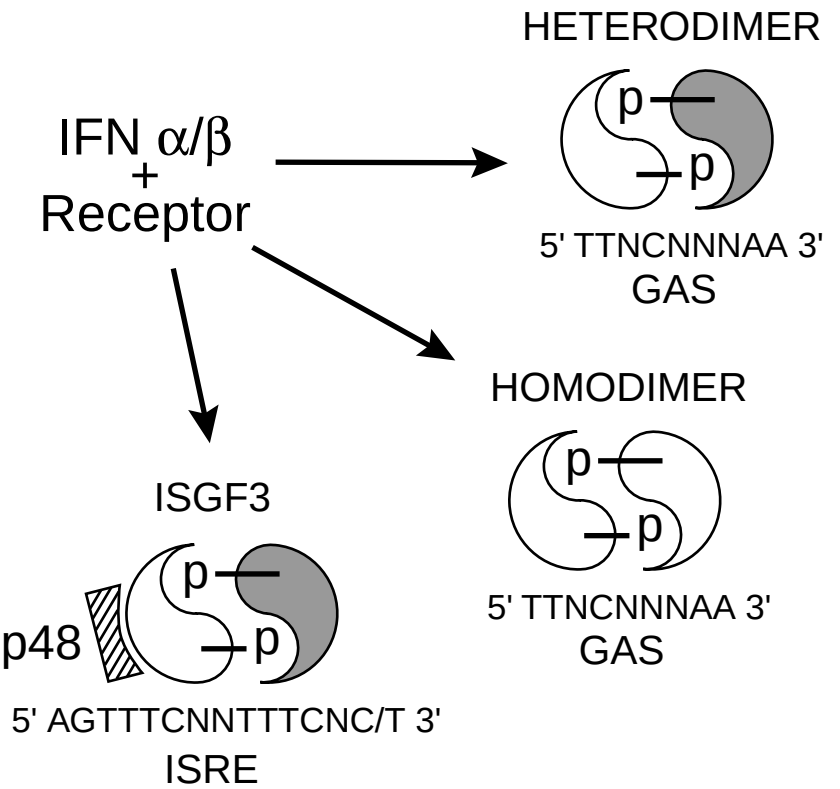


Figure 4 Transcription factors formed at the liganded interferon (IFN)α/β receptor and their DNA recognition elements. Signal transducer and activator of transcription (STAT) 1 and STAT2 heterodimers and STAT1 homodimers bind to identical gamma-activated sequence (GAS) elements, whereas interferon-stimulated gene factor 3 (ISGF3) binds to interferon-stimulated regulatory elements (ISREs).

dimers that are formed in response to many different ligands, including the IFNs, bind to GAS elements whose sequences determine the specificity of the interactions (74). Little current information is available on the regions of STAT dimers that are in contact with the DNA of GAS elements.

Xu et al (75) and Vinkemeier et al (76) have found that the N-terminal domain of STATs 1 and 4 are required for the respective pairs of homodimers to cooperate in binding to tandem GAS sites, which are found in the promoters of some genes that are induced in response to activation of these STATs, for example, the IFNγ gene (75) and the *mig* gene (77). Other genes, for example,

6–16 (78), also have tandem ISREs, and it may be that the cooperative binding of two ISGF3 moieties is required for their optimum expression.

Recent publications have established important connections between STATs 1 and 2 and the CREB-binding protein (CBP)/p300 transcription factors. Zhang et al (79) showed that the N-terminal region of STAT1 interacts with the cyclic-AMP response element binding protein (CREB)–binding domain of CBP/p300 and that the C-terminal region of STAT1 interacts with the domain of CBP/p300, which also binds to the adenovirus protein E1A. Furthermore, both unphosphorylated STAT1 monomers and the phosphorylated STAT1 dimers formed in response to IFNγ are competent to bind to CBP/p300. In transient expression assays, cotransfection of CBP/p300 potentiated and E1A inhibited the activation of a GAS-driven reporter in response to IFNγ. Similarly, Horvai et al (80) showed that the STAT1 and AP1/ets factors that are activated by *Ras*-dependent signaling compete for the limiting amounts of CBP/p300 that each requires for activity. Impressively, microinjection of antibodies directed against CBP/p300 blocks transcriptional responses to IFNγ. Furthermore, Rutherford et al (81) found that the Ets-1 protein of mice binds to an ISRE and may negatively regulate activation by ISGF3. It remains to be seen whether CBP/p300 is required for the transcriptional response to STAT1 homodimers formed in response to IFNα/β, but Bhattacharya et al (82) have shown that CBP/p300 also binds to the C-terminal region of STAT2 and that blockade of this interaction by the adenovirus E1A protein inhibits ISRE-mediated responses to IFNα/β. These fascinating studies provide the first indication of how STATs may interact with the transcriptional machinery.

The mitogen-activated protein kinase (MAPK) cascade is activated by IFNα/β, and the effect of this activation on signaling has been explored. David et al (35) found that ERK2 (the 42-kDa MAPK) binds to a glutathione S-transferase (GST) fusion protein containing the membrane-proximal 50 residues of the cytoplasmic domain of IFNAR1 but not to the full-length cytoplasmic domain of $\sim$100 residues. However, there was association between ERK2 and full-length IFNAR1 in vivo. Treatment of cells with IFNβ induced the tyrosine phosphorylation and activation of ERK2 and caused it to associate with STAT1, as judged by coprecipitation. Furthermore, expression of a dominant negative form of MAPK inhibited IFNβ-induced transcription in a transient cotransfection assay employing an ISRE-driven reporter. It is tempting to connect these observations with those of Wen et al (34), who showed that serine 727 of STAT1, which lies in a MAPK consensus site, is phosphorylated in response to IFNγ and that this phosphorylation increases the response of a GAS-driven promoter to IFNγ. Unfortunately, the connection is not clear. It has not yet been shown that STAT1 is phosphorylated on serine in response to IFNα/β. Wen et al (34) have argued that such phosphorylation is unlikely

to be important for the activation of ISRE-driven genes because STAT1β, an alternatively spliced form of STAT1 lacking serine 727, can form ISGF3 and drive the expression of such genes, albeit not as well as STAT1α (83). This is presumably because STAT2 provides a potent transactivation domain. STAT2 does not contain a serine residue in a MAPK consensus site and is not known to be phosphorylated in response to IFN. Thus, the basis of the cross talk between the IFNα/β and MAPK pathways requires further clarification. In more recent work, Stancato et al (84) showed that Raf1, which lies between Ras and ERK2 in the MAPK cascade, is activated by IFNβ in a manner that does not require Ras but does require JAK1. Furthermore, Raf1 activated by IFNβ can be coprecipitated with either JAK1 or Tyk2.

IFNα/β treatment causes phosphorylation and activation of cytosolic phospholipase A_2 ($CPLA_2$), which requires JAK1. Furthermore, JAK1 and $CPLA_2$ can be coprecipitated (85). Inhibitors of $CPLA_2$ inhibit IFNα/β-induced expression of ISRE-driven genes, but not of a GAS-driven gene, which implies that $CPLA_2$ is somehow required for the formation of ISGF3 but not of STAT1 homodimer. The basis for this interesting effect remains to be discovered.

The insulin receptor substrate-1 (IRS1) is phosphorylated on tyrosine residues in response to IFNα/β and, in this state, plays its usual role in bringing phosphatidylinositol 3′-kinase to receptors for activation by tyrosine phosphorylation, by engaging the SH2 domains of the p85 regulatory subunit (86). Burfoot et al (87) found that this activation of IRS1 depends on both JAK1 and Tyk2, thus requiring full function of the IFNα/β receptor. However, the physiological significance of this activation is unclear, because cells lacking both IRS1 and IRS2 show little difference from cells that express these proteins in cell growth inhibition in response to IFNα/β or -γ (87). More experiments need to be done to test the requirement for phosphatidylinositol 3′-kinase in a variety of IFN responses.

Interferon β: A Subtype-Specific Pathway

Humans have at least 12 functional IFNαs, a single antigenically distinct IFNβ, and a related IFNω (88), whereas other species have multiple IFNβ subtypes. The human IFNαs are synthesized predominantly by a subset of lymphocytes, and IFNβ is made by fibroblasts. Work with different IFNα subtypes, produced by recombinant DNA technology and by purification of natural leukocyte IFNs, has revealed substantial differences in their specific antiviral activities, in the ratios of antiviral to antiproliferative activities, and in a number of additional functions (89–92). A priori, it might be expected that different cell types might respond differentially to the different IFNs, but to date there is no clear evidence of this (for example, see Reference 91). The various IFNα/βs appear to interact with the same receptor and to have antiviral, antiproliferative, and immunomodulatory activities in a number of cell types. The functional significance of the

multiple species, and how functional differences are mediated through apparently identical receptors, remain intriguing questions in this area of research. Interesting differences are emerging, both with respect to IFN-receptor interactions and the induced mRNAs. Mutant cells in the U1 complementation group, lacking Tyk2, are completely defective in response to a purified mixture of natural IFNαs or to recombinant IFNα1 or -α2; nevertheless, they retain partial responses to IFNβ (56) and IFNα8 (91). How the residual IFNβ response is mediated is not yet known, but, importantly, it is not seen in mutant cell lines lacking JAK1, STAT1, or STAT2. It is likely, therefore, to be mediated through JAK-STAT pathways but without an absolute requirement for Tyk2 in the receptor complex. Consistent with this model, IFNβ engages the receptor in a distinct fashion. The groups of Revel and Colamonici first noted the rapid, transient tyrosine phosphorylation of a receptor-associated 100-kDa protein in response to IFNβ but not to IFNα (93, 94). This protein has recently been identified as the IFNAR2c subunit of the receptor (95, 96). Importantly, IFNAR2c is phosphorylated apparently equivalently in response to IFNα or -β, the difference lying in its ability to coprecipitate with IFNAR1 from the complex with IFNβ but not IFNα (95, 96). It remains to be established whether this intriguing finding reflects a tighter association of the two subunits in the β versus the α complex, which might in turn reflect structural differences that, if transmitted through the membrane to the intracytoplasmic domains, mediate a differential response. Importantly, βR1, a gene transcribed preferentially in response to IFNβ, has been discovered (97). The isolation and characterization of the corresponding promoter should, through the identification of known and novel motifs, provide evidence for the involvement of known signaling pathways and experimental handles to investigate unknown ones.

Modulation of IFN Responses

Proteins of the IRF family, such as IRF2 (98), ICSBP (99), and ICSAT (100), bind to ISREs and negatively regulate expression of the associated genes. These repressors may help to prevent the expression of ISGs in the absence of IFN, to down-regulate the induced response, or both. Treatment of cells with IFN in the presence of protein synthesis inhibitors prolongs ISG transcription (101, 102), indicating that some IFN-induced proteins may help to shut off the response. These may be repressors or other types of inhibitors (see below). A mutant cell line with IFN-independent constitutive expression of ISGs has been isolated. There is little or no defect in shutting off the response to IFNα/β in these cells, suggesting that these two aspects of negative regulation can be distinguished (DW Leaman, A Salvekar, R Patel, GC Sen & GR Stark, unpublished data).

The amount of active STAT1 can be reduced by dephosphorylation (103, 104). Proteasome-mediated degradation may also have a role (105), though this aspect

is controversial (104). Inhibition of phosphatases by potent agents such as peroxyvanadate stabilizes the ligand-induced phosphorylation of STATs (106, 107) and can also lead, more slowly, to the ligand-independent accumulation of phosphorylated STATs (107). The inhibited phosphatases may operate on phosphorylated STATs in the nucleus or on phosphorylated JAKs or receptor subunits at the plasma membrane (103, 106). Phosphatases with SH2 domains are especially good candidates for the latter function, and SHP-1 has been implicated in this function in hematopoietic cells (108, 109). Other phosphatases would have to assume this role in most other cell types, where SHP-1 is not expressed. Phorbol esters, which inhibit signaling in response to IFNα/β, can do so by activating one or more tyrosine phosphatases that selectively dephosphorylate Tyk2 but not JAK1 or IFNAR1 (110). Decreased availability of p48 may also play a role (111). The ISGF3-mediated response to IFNα/β is initiated by the rapid formation of ISGF3, but at least for some genes, it is likely to be sustained by IRF1 (69). In human fibroblasts, the level of ISGF3 declines over the course of a few hours and returns to a basal level after 4 h. However, at this time, the transcription rate of the 6-16 gene is still at a maximum, coincident with the maximum in the IFN-induced expression of IRF1 (69). The eventual return of 6-16 transcription to a basal level, in about 8 h, corresponds to the decline of IRF1. By studying mice null for expression of p48, IRF1, or both, Kimura et al (112) have shown that p48 and IRF1 do not have redundant functions but instead complement each other in the responses to both IFNα/β and IFNγ.

FUNCTIONS INDUCED BY INTERFERONS

Antiviral Activities

The ability of IFNs to confer an antiviral state on cells is their defining activity as well as the fundamental property that allowed their discovery (113). IFNs are essential for the survival of higher vertebrates because they provide an early line of defense against viral infections—hours to days before immune responses. This vital role has been demonstrated by the exquisite sensitivity to virus infections of mice lacking both IFNα/β and γ receptors (114). Multiple, redundant pathways have evolved to combat different types of viruses and the various compensatory defense mechanisms that different viruses have evolved (see below). Any stage in virus replication appears to be fair game for inhibition by IFNs (115), including entry and/or uncoating [simian virus 40 (SV40), retroviruses], transcription [influenza virus, vesicular stomatitis virus (VSV)], RNA stability (picornaviruses), initiation of translation (reoviruses, adenovirus, vaccinia), maturation, and assembly and release (retroviruses, VSV).

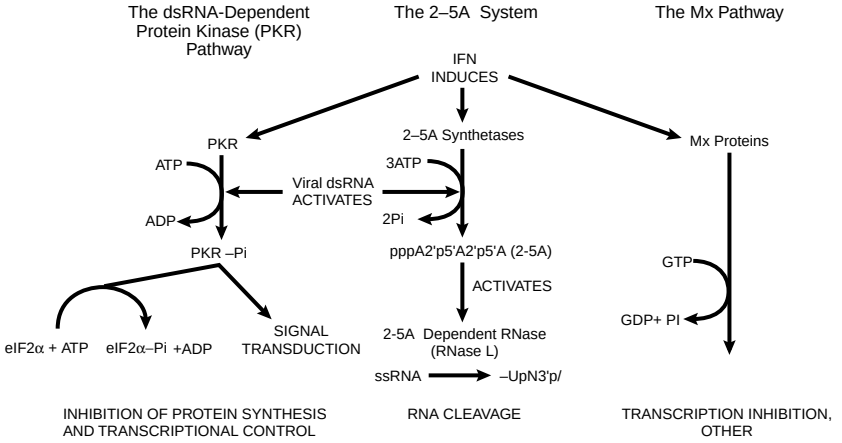


Figure 5 Antiviral mechanisms of interferon (IFN) action.

PKR The best-characterized IFN-induced antiviral pathways utilize the dsRNA-dependent protein kinase (PKR), the 2-5A system, and the Mx proteins (Figure 5). PKR is a serine-threonine kinase with multiple functions in control of transcription and translation (116, 117). PKR is normally inactive, but on binding to dsRNA, it undergoes autophosphorylation and subsequent dsRNA-independent phosphorylation of substrates. Two conserved dsRNA-binding motifs are present in the N-terminal regulatory half of PKR. The first mediates dsRNA-binding activity and includes residues critical for binding to dsRNA, highly conserved within a large family of dsRNA-binding proteins. No RNA sequence specificity is required for dsRNA to bind to PKR. The resulting conformational change in the enzyme probably unmasks its C-terminal catalytic domain (118). The antiviral effect of PKR is due to its phosphorylation of the alpha subunit of initiation factor eIF2. This phosphorylation results in the formation of an inactive complex that involves eIF2-GDP and the recycling factor eIF2B, resulting in rapid inhibition of translation. Apoptosis may also play a role in the antiviral effect of PKR (see below). Overexpression of PKR leads to the suppression of encephalomyocarditis virus (EMCV) replication in cultured cells (119). In addition, a dominant negative PKR mutant or an antisense PKR cDNA construct suppresses the anti-EMCV effect of IFNα and IFNγ in promonocytic U-937 cells (120). Poly(I):poly(C) or IFNγ treatment extends the survival of wild-type but not PKR-null mice after infection with EMCV (121). In contrast, redundancy in the antiviral pathways is apparent because IFNα extends to the same extent the survival of wild-type and PKR-null mice after EMCV infection.

THE 2-5A SYSTEM This system is a multienzyme pathway (Figure 5) in which IFN-inducible 2-5A synthetases are stimulated by dsRNAs, often of viral origin, to produce a series of short, 2′,5′-oligoadenylates (2-5A) that activate the 2-5A–dependent RNase L (122, 123). Activation of this pathway leads to extensive cleavage of single-stranded RNA (124–126). The 2-5A synthetases (40, 46, 67, 69, 71, and 100 kDa) are encoded by multiple genes and reside in different parts of the cell (127–130). 2-5A binds to inactive, monomeric RNase L, inducing the formation of the homodimeric, active enzyme (131–133). The activation of RNase L is reversible (134). Its N-terminal half is a repressor that contains a repeated P-loop motif and nine ankyrin repeats, both involved in 2-5A binding. The C-terminal half contains a region of protein kinase homology, a cysteine-rich domain, and the ribonuclease domain (135, 136). The isolated C-terminal half of RNase L cleaves RNA in the absence of 2-5A (137). There are striking similarities between RNase L and IRE1p, a yeast endoribonuclease that functions in Hac1 mRNA splicing in the unfolded protein response (138). An intriguing possibility is that RNase L might exist as a member of a family of regulated nucleases with diverse functions in different organisms.

The functions of the 2-5A system have been explored through genetic manipulation of RNase L. Cells expressing a dominant negative derivative were defective in expressing the anti-EMCV and antiproliferative activities of IFNα/β, whereas overexpression of RNase L blocked vaccinia virus and HIV-1 replication (136, 139; RK Maitra & RH Silverman, unpublished data). RNase L–null mice are deficient in both the anti-EMCV effect of IFNα and in several apoptotic pathways (140). Although IFNα treatment extended the survival of both wild-type and RNase L–null mice after EMCV infection, the RNase L–null mice died several days earlier, lending support to cell culture studies linking the 2-5A system to the anti-EMCV effect of IFN (123). The ability of RNase L to be activated by small molecules opens up possibilities for drug design and development. In one such example, RNase L was recruited by 2-5A–antisense oligonucleotides to cleave respiratory syncytial virus M2 RNA selectively, thus blocking viral replication in human tracheal epithelial cells (141, 142). A mammalian 2-5A system has also been cloned in transgenic tobacco plants, resulting in resistance to several different viruses (143, 144).

THE MX PROTEINS Mx proteins are IFN-inducible, high-abundance 70- to 80-kDa GTPases in the dynamin superfamily (145, 146). Mx proteins and dynamins self-assemble into horseshoe- and ring-shaped helices and other helical structures (147–149). Human MxA forms tight oligomeric complexes in cell-free systems and in intact cells (150, 151). The Mx proteins interfere with viral replication, impairing the growth of influenza and other negative-strand RNA viruses at the level of viral transcription and at other steps. The

murine nuclear protein Mx1 suppresses the growth of influenza, Thogoto, and tick-born Dhori viruses, and the human cytoplasmic protein MxA inhibits the growth of influenza, VSV, measles, Thogoto, bunya, phlebo, hanta, and human parainfluenza 3, but not Dhori viruses (152–157). Mutant forms of Mx proteins lacking the ability to bind or hydrolyze GTP fail to suppress viral replication. However, the binding, not the hydrolysis, of GTP is required to inhibit VSV transcription by MxA in vitro (158, 159). Mx proteins are believed to interfere with the trafficking or activity of viral polymerases (160). Furthermore, MxA specifically binds to Thogoto-virus ribonucleoprotein complex (O Haller, personal communication). Murine Mx1 inhibits the primary transcription of influenza virus, whereas human MxA acts in the cytoplasm to inhibit a later step in the viral life cycle (161). Although PKR and Mx genes are induced preferentially by type I IFNs, 2-5A-synthetase and RNase L are induced by both types (116, 135, 162, 163). Also, the induction by IFNγ (but not IFNα/β) of nitric oxide synthase in mouse macrophages inhibits the growth of ectromelia, vaccinia, and HSV-1 viruses (164). Therefore, different antiviral pathways may be induced in different cell types, depending on the type of IFN involved.

Many IFN-induced proteins are poorly characterized, and some of these are very likely to possess antiviral activity. For instance, expression of the IFN-inducible 9-27 protein led to a partial inhibition of VSV replication (165). Clearly, the enormous selective pressures imposed by viruses have resulted in a rich and diverse set of antiviral pathways.

VIRAL INHIBITION OF THE IFN RESPONSE Hardly surprising, viruses fight back, not only against host defenses in general (166–169) but also against the IFN systems in particular, both through novel mechanisms and by subverting host systems through the synthesis of novel proteins and proteins that mimic and thus interfere with host proteins (e.g. the IFN receptors; see References 169, 170). There is evidence for the inhibition of the 2-5A–dependent RNase L in response to EMC infection (171) and for a cellular protein inhibitor of RNase L (172), but the most extensively studied examples involve the inhibition of PKR. At least four different mechanisms are used, including inhibitory viral RNA, inhibitory viral or cellular proteins, and proteolytic cleavage. Best studied is the adenovirus virus-associated (VA) RNA, which binds to but does not activate PKR (173). An important fact is that mutant viruses lacking VA RNA are more sensitive to IFN-mediated inhibition (174). Epstein-Barr virus-encoded small nonpolyadenylated RNAs (EBER) may perform a similar function for Epstein-Barr (EB) virus (175), although an EBER-negative strain shows no obviously enhanced sensitivity to IFNs in vitro (176). Examples of proteins that sequester the viral dsRNA activators of PKR are the reovirus sigma 3 capsid protein (177)

and the vaccinia virus E3L protein (178). The HIV transcriptional transactivator (TAT) (179, 180) and hepatitis C virus NS5A (181) proteins appear to inhibit by interacting with PKR directly. In response to EMC infection, proteolytic cleavage of PKR in poliovirus-infected cells and sequestration of the enzyme occur (182, 183). Particularly interesting are the cellular protein systems that inhibit PKR in response to influenza virus infection. p58(IPK), a cellular protein inhibitor of PKR, is inhibited by I-p58(IPK), which is apparently inactivated in response to infection. I-P58(IPK) has recently been identified as the molecular chaperone hsp40; this identification revealed that the influenza virus regulates PKR activity by recruiting a cellular stress protein (184). Both cells and viruses have developed elegant mechanisms to control PKR, which shows the importance of this enzyme in controlling cellular functions (see below) and virus replication. As a variant on this theme, SV40 can restore efficient translation in cells, despite the elevated levels of phosphorylated eIF2α that result from activating PKR, because the translational rescue mediated by the SV40 large T antigen occurs downstream of the phosphorylation of eIF2α (185).

Cell death in response to virus infection may be mediated by apoptosis as well as necrosis (186). Interestingly, cells from mice lacking the 2-5A–dependent RNase L or PKR show defects in apoptosis (140, 187), consistent with a possible role for these enzymes in virus-induced, IFN-mediated cell death. Poxviruses produce CrmA, and the Kaposi's sarcoma herpes virus produces FLIPs (inhibitors of the apoptotic ICE and FLICE proteases, respectively), presumably to suppress host-cell suicide and inflammatory responses (188, 189). Other viral anti-apoptotic genes resemble the mammalian bcl-2 gene, which suppresses apoptosis (186, 190).

The adenovirus E1A and human papilloma virus E6 and E7 proteins inhibit the production and action of IFN at the level of transcription (66, 191–194). For E1A, the effect on the transcription of ISGs is mediated, in part at least, through a reduction in functional p48 (192, 193) and probably also by sequestering p300/CBP, required for transcriptional activation through STAT2 (82). A similar inhibition at a transcriptional level of IFN production and action is mediated by the Kaposi's sarcoma herpes virus through the production of an inhibitory mimic of IRF (169). In additional strategies, the poxviruses produce soluble IFNα and IFNγ receptors (116), and the EB virus generates an IL-10 analog. Interestingly, the type I IFN receptor mimic produced by the vaccinia virus shows a wide species specificity, consistent with the broad host range of the virus (195). The IL-10 analog probably performs a dual function for the virus, inhibiting the production of IFNγ and activating the B lymphocytes necessary for virus replication (196).

The IFN systems are subject to cellular control during development and differentiation and are subject to inhibition by viruses. The multiple mechanisms

involved emphasize the importance of these systems to both cells and viruses. Conversely, the IFN systems are not, of course, the only host defense systems against which viruses retaliate. Indeed, to know which host defenses are important, viruses must be investigated.

Inhibition of Cell Growth

IFNs inhibit cell growth and control apoptosis, activities that affect the suppression of cancer and infection. Genes have been identified that are important for the apoptotic, but not the growth inhibitory, effects of IFNγ (197). Therefore, these two activities of IFNs are considered separate but related topics.

Different cells in culture exhibit varying degrees of sensitivity to the antiproliferative activity of IFNs. In some cases, growth arrest may be due to differentiation, particularly when IFNs are used in combination with other agents such as retinoids (198, 199). Specific IFN-induced gene products have not been linked directly to antiproliferative activity. However, IFNα has been shown to target specific components of the cell-cycle control apparatus, including c-myc, pRB, cyclin D3, and cdc25A (200–203). Lymphoblastoid Daudi cells are exquisitely sensitive to the antiproliferative effects of IFNα, which lead to a rapid shutdown of c-myc transcription, possibly through a decrease in the activity of the transcription factor E2F (202). Cells expressing a transdominant mutant of PKR fail to suppress c-myc in response to IFN, although the phosphorylation of pRB is suppressed (204). PKR may play a subtle role in cell growth regulation. Early-passage embryo fibroblasts (MEFs) established from PKR-null mice achieve saturation densities similar to those of wild-type MEFs, whereas PKR-null cells consistently achieve higher saturation densities beyond five passages. The doubling times of wild-type and PKR-null cells do not differ appreciably between early and late passages, however (S Der & BRG Williams, unpublished data). This phenotype is similar to the increased saturation densities described for MEFs derived from p53-null or p21/WAF1-null mice (205) and may result from increased resistance to apoptosis, induced by growth-factor deprivation in the absence of PKR.

The phosphorylation of pRB by IFN is suppressed by the inhibition of cdk4 and cdk6. This inhibition is achieved through the suppression of cyclin D3 and by preventing the activation of cdk2-cyclinA and cdk-cyclinE, thereby inhibiting the phosphatase cdc25A (203). This mechanism of growth suppression is distinct from that of other growth-inhibitory cytokines such as TGFb because it does not appear to involve the induction of cdk inhibitors such as p21, p27, and $p57^{kip}$. Cell-type differences clearly complicate any mechanistic understanding of the antiproliferative effects of IFN. For example, in contrast to the Daudi cells discussed above, the induction of p21-cdk2 has been correlated with growth inhibition of the prostate cancer cell line DU145 by IFNα (206).

Control of Apoptosis

IFNs are essential for host responses to viruses and some other microbial pathogens, events that often culminate in apoptosis. However, development in the mouse proceeds normally in the absence of functional IFNα/β and IFNγ receptors (114). IFNs have either pro- or anti-apoptotic activities, depending on factors such as the state of cell differentiation. For instance, IFNγ either induced or inhibited the apoptosis of murine pre-B cells or B chronic lymphocytic leukemia cells, respectively (207–209). Similarly, IFNγ promoted either proliferation or apoptosis in malignant human T cells, depending on the presence or absence of serum and the levels of the IFNγ receptor (210). The involvement of IFNs in apoptosis is interwoven with the roles of other modulators of apoptosis and the enzymes they regulate. For example, dsRNA produced during viral infections, and lipopolysaccharide (LPS), an endotoxin of gram-negative bacteria, are potent inducers of apoptosis. Interestingly, either dsRNA or LPS induces the synthesis of IFNs. The induction of IFNγ by LPS requires the activity of caspase-1 (ICE) to process the IFNγ-inducing factor IGIF or IL-18 (211, 212). Similarly, dsRNA induces the production of IFNα/β and is a pro-apoptotic agent (187).

Investigations into molecular pathways mediating IFN-induced apoptosis have focused either on the antiviral enzymes (see below) or on identifying other proteins in the pathway. Several genes have been cloned that, when down-regulated, suppress the growth inhibitory or apoptotic activities of IFNγ in HeLa cells. Five novel genes for death-associated proteins, DAP-1 to -5, and two other proteins (thioredoxin and the protease cathepsin D) play a role in these processes (197, 213–218). Down-regulation of DAP-1 (a proline-rich protein), DAP-2 (DAP kinase), DAP-3, or cathepsin D with antisense RNAs blocked the apoptotic activity but not the cytostatic effect of IFNγ. DAP-2, a calmodulin-dependent protein kinase with a consensus death domain, localizes to the cytoskeleton, and its expression is lost frequently in human tumors (218, 219). DAP-2 is a tumor suppressor gene that couples the control of apoptosis to metastasis (219a). DAP-5 was identified from a partial cDNA encoding a dominant negative protein related to the protein-synthesis initiation factor eIF4G (217). It will be interesting to determine how all of these proteins interact with each other and with other intracellular apoptotic factors.

Effects of IFNs on the Immune System

The immunomodulatory actions of IFNs have been studied extensively, but because of space limitation, they cannot be discussed in detail here. Several reviews on IFNα/β and IFNγ biology have been published recently, and the reader is referred to these for more details (12, 115, 220, 221). Here we identify major recent advances in understanding the roles of IFNs in promoting

immune responses, and we provide examples of how the actions of IFNα/β and IFNγ diverge. IFNs are known to profoundly affect nearly all phases of innate and adaptive immune responses. Within the IFN family, IFNγ plays the predominant immunomodulatory role. It is produced by a restricted set of immune cells (T cells and natural killer cells) in response to immune and/or inflammatory stimuli and functions to stimulate the development and actions of immune effector cells. The immunomodulatory actions of IFNα/β are more restricted: They are directed largely at promoting responses that provide the host with adaptive immune response mechanisms to resist viral infection.

IFN, ANTIGEN PROCESSING AND PRESENTATION, AND DEVELOPMENT OF CD8$^+$ T-CELL RESPONSES One unarguable role of IFNs in promoting protective immune responses is their ability to regulate the expression of proteins encoded in the major histocompatibility complex (MHC). All IFN family members share the ability to enhance the expression of MHC class I proteins and thereby to promote the development of CD8$^+$ T-cell responses (221). This expression is known to be driven by IRF1, the transcription factor predominantly responsible for activating MHC class I gene transcription (222, 223). Cells from mice with targeted mutations in either the IFNγ or IFNα/β receptor systems, STAT1, PKR, or IRF1 fail to up-regulate MHC class I proteins on their surface in response to stimulation by the appropriate IFN. In contrast, IFNγ is uniquely capable of inducing the expression of MHC class II proteins on cells, thereby promoting enhanced CD4$^+$ T-cell responses (221, 224). This response depends on a distinct transactivating factor, CIITA. Cells from human patients with the rare abnormality bare-lymphocyte syndrome, characterized by the absence of CIITA, fail to express MHC class II proteins either constitutively or following exposure to IFNγ. IFNγ induces MHC class II protein expression in a wide variety of different cell types, such as mononuclear phagocytes, endothelial cells, and epithelial cells, but it inhibits IL-4–dependent class II expression on B cells (225). The molecular basis for this discordant effect is unknown.

IFNs also play an important role in antigen processing by regulating the expression of many proteins required to generate antigenic peptides. IFNγ modifies the activity of proteasomes by modulating the expression of both enzymatic and nonenzymatic components (221, 226). The proteasome is a multisubunit enzyme complex that is responsible for the generation of all peptides that bind to MHC class I proteins. In unstimulated cells, it contains three enzymatic subunits: x, y, and z. However, following treatment of cells with IFNγ, transcription of the x, y, and z genes decreases, and transcription of three additional genes encoding different enzymatic proteasome subunits, LMP2, LMP7, and MECL1, increases. This leads to the formation of different proteasomes

containing these subunits and possessing a different substrate specificity, thereby altering the types of peptides produced and eventually presented to the immune system. IFNγ also induces the expression of a nonenzymatic proteasome subunit, PA28 (also known as the 11S regulator), which binds to proteasome enzyme components and alters their specificity (227, 228). Finally, IFNγ increases the expression of TAP1 and TAP2, which transfer peptides generated by the proteasome in the cytoplasm into the endoplasmic reticulum, where they bind to nascent MHC class I chains (229, 230). Thus, IFNs enhance immunogenicity by increasing the quantity and repertoire of peptides displayed in association with MHC class I proteins.

IFNγ AND DEVELOPMENT OF THE CD4$^+$ HELPER T-CELL PHENOTYPE Activated human and murine CD4$^+$ T cells can differentiate into two polarized subsets, defined by the cytokines they produce when stimulated (231). In mice, T helper 1 (Th1) cells have the selective ability to synthesize IFNγ, lymphotoxin (LT), and IL-2 and to promote cell-mediated immunity and delayed type hypersensitivity (DTH) responses. In contrast, murine Th2 cells selectively produce IL-4, IL-5, IL-6, and IL-10 and thereby facilitate antibody production and the development of humoral immune responses. IFNγ has an important effect on Th1 cell development. In vitro, antibody-mediated neutralization of IFNγ greatly reduces the development of Th1 cells and augments the development of Th2 cells (232). Similar effects are seen in mice lacking the ability to respond to IFNγ, i.e. STAT1-null mice. However, administration of exogenous IFNγ, in vitro or in vivo, does not drive a Th1 response. Thus, IFNγ is necessary but not sufficient for Th1 development.

IFNγ plays a dual role in this process. First, it facilitates Th1 production by enhancing the synthesis of IL-12 in antigen-presenting cells (233–235). IL-12 is the proximal effector that drives developing CD4$^+$ T cells to become Th1 cells (232, 236). In addition, IFNγ maintains expression of the β2 subunit of the IL-12 receptor on developing CD4$^+$ T cells, thereby preserving their capacity to respond to IL-12 (237). Second, IFNγ blocks the development of Th2 cells by inhibiting the production of IL-4, which is required for Th2 formation (238), and by preventing Th2-cell proliferation (239). Th1 cells are not affected in this manner because they become insensitive to IFNγ as a result of IFNγ-dependent down-regulation of the expression of IFNGR2 (36, 37).

IFN, MACROPHAGE ACTIVATION, AND CELLULAR IMMUNITY Macrophages function as a key effector cell population in innate and adaptive immune responses. To carry out these functions, they must first become activated, a process involving a reversible series of biochemical and functional alterations that provide them with enhanced cytocidal activities (240). Through the use

of neutralizing IFNγ-specific monoclonal antibodies and gene-targeted mice, it has been possible to establish unequivocally the predominant role played by IFNγ in generating activated macrophages, both in vitro and in vivo (241–243). Importantly, the macrophage-activating activity of IFNγ is not provided by IFNα/β. Supporting data come from studies demonstrating that IFNγ-unresponsive mice or humans (i.e. IFNγ-null, IFNγ receptor–null, or STAT1-null mice or patients with inactivating mutations in the IFNGR1 gene) are highly susceptible to infection with a variety of microbial pathogens such as *Listeria monocytogenes*, *Toxoplasma gondii*, *Leishmania major*, and several different strains of *Mycobacteria* (41, 242–245). Increased susceptibility to infection occurs in IFNγ-unresponsive hosts despite their ability to maintain an unaltered capacity to produce and respond to IFNα/β.

Activated macrophages use a variety of IFNγ-induced mechanisms to kill microbial targets. Two of the most important involve the production of reactive oxygen and reactive nitrogen intermediates. Reactive oxygen intermediates are generated as a result of the IFNγ-induced assembly of NADPH oxidase, formed as a result of the induced translocation of two cytosolic enzyme subunits to the plasma membrane, where they combine with a membrane-associated electron transport chain component, cytochrome *b*558 (246). This enzyme effects a one-electron transfer to oxygen, producing superoxide anion, which, in turn, is used to generate additional toxic oxygen compounds such as hydrogen peroxide, hydroxyl radicals, and singlet oxygen. Reactive nitrogen intermediates, particularly nitric oxide (NO), are generated in murine macrophages as a result of the IFNγ-dependent transcription of the gene encoding the inducible form of nitric oxide synthase (iNOS), which catalyzes the formation of large amounts of NO (247). NO is thought to kill target cells by one of two mechanisms. First, it can form an iron-nitrosyl complex with the Fe-S groups of aconitase, complex I and complex II, thereby inactivating the mitochondrial electron transport chain. Alternatively, NO can react with superoxide anion to form peroxynitrite, which decays rapidly to form highly toxic hydroxyl radicals. Although iNOS is induced in murine macrophages in an IFNγ-dependent manner, it is not induced in human mononuclear phagocytes exposed to the same stimuli. The molecular basis for this difference has not yet been defined.

IFN AND HUMORAL IMMUNITY IFNs play complex and sometimes conflicting roles in regulating humoral immunity. Most analyses have attempted to define the influence of IFNγ in the process, although more recent observations suggest that IFNα/β may also induce many of the same biological effects. IFNs exert their effects either indirectly (as described above), by regulating the development of specific T helper cell subsets, or directly at the level of B cells. In the latter case, IFNs are predominantly responsible for regulating three

specialized B-cell functions: development and proliferation, immunoglobulin (Ig) secretion, and Ig heavy-chain switching.

The best-characterized action of IFNs directed toward B cells is their ability to influence Ig heavy-chain switching. Ig class switching is significant because the different Ig isotypes promote distinct effector functions in the host. By favoring the production of certain Ig isotypes while inhibiting the production of others, IFNs can facilitate interactions between the humoral and cellular effector limbs of the immune response and increase the host defense against certain bacteria and viruses. In vitro, IFNγ is able to direct immunoglobulin class switching from IgM to the IgG2a subtype in LPS-stimulated murine B cells (248) and to IgG2a and IgG3 in murine B cells that have been stimulated with activated T cells (249). Moreover, IFNγ blocks IL-4–induced Ig class switching in murine B cells from IgM to IgG1 or IgE (250). The validity of these observations has been tested stringently by injecting mice with polyclonal anti-IgD serum, a polyclonal activator of B cells. These mice produced large quantities of IgG1 and IgE. However, when IFNγ was administered prior to anti-IgD treatment, the mice produced high levels of IgG2a and decreased levels of IgG1. Thus, IFNγ is clearly an important regulator of Ig class switching in vivo.

A role for type I IFNs in this process has also been identified (251). Of particular importance are experiments using mice that lack receptors for IFNγ, IFNα/β, or both (114). The mice were infected with lymphocytic choriomeningitis virus (LCMV), and the profiles of the LCMV-specific antibodies generated were determined. Comparable levels of LCMV-specific IgG2a antibodies were observed in the sera of normal mice and of mice unresponsive to either IFNγ or IFNα/β. In contrast, IgG2a antibodies were not produced in mice lacking responses to both types of IFN. These results demonstrate that if induced during the immune response, IFNs α/β can indeed function in a manner redundant to IFNγ in effecting Ig class switching.

ADDITIONAL FUNCTIONS OF PROTEINS INVOLVED IN IFN RESPONSES

JAKs

JAKs can auto- and transphosphorylate, and it is reasonable to assume that they phosphorylate the receptors and the proteins recruited to them, foremost among which are STATs. The interaction of SH2 domains with receptor subunit phosphotyrosine motifs clearly plays a major role in recruitment. But it is increasingly unlikely that this is the whole story, and recruitment directly by JAKs is an interesting alternative. For example, it appears possible that signaling by growth hormone can be achieved with a receptor entirely lacking phosphotyrosine

motifs (252). Fujitani et al (253) have presented evidence for the recruitment of STAT 5 through JAK2, and a number of additional JAK-signaling component interactions have been reported (see below). More generally, JAK1 and JAK2 are present in the cell nucleus as well as in the cytoplasm and at membranes (254; A Ziemiecki, personal communication). Initial results with a dominant negative derivative of JAK1 raise the possibility of a constitutive requirement for JAKs early in zebra fish development (255). JAK3-null mice show no obvious defect in early development (256, 257). JAK1-null mice, however, are runted, fail to nurse, and die perinatally. They also appear to have a sensory neuron defect, which includes a failure of explanted dorsal root ganglion neurons to survive when cultured in the presence of IL-6, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M (OSM), or cardiotrophin 1 (CT-1), all neurotrophic factors that signal to the JAKs through the IL-6 receptor family (S Rodig & R Schreiber, unpublished data). JAK2-null mice die early in embryogenesis, consistent with a failure of hematopoeisis (E Parganas & JN Ihle, personal communication). Of course, it remains to be established that any of the defects in the knockout mice reflect a requirement for additional JAK functions. That said, it has become increasingly clear that just as JAKs may not be the only mediators of STAT activation, STATs are not the only targets of activated JAKs. Evidence for this comes from both protein association data and functional experiments. For example, growth hormone, IL-11, and OSM all promote the association of JAK2 with Shc and Grb2 (258–260). Work with a JAK2-null cell line has established that the phosphorylation of Shc in response to growth hormone depends on JAK2 (261), consistent with a requirement for cotransfected JAK2 to achieve MAP kinase activation in response to activation of a transfected growth hormone receptor (262). Raf1 associates with JAK2 when coexpressed in the baculovirus system and in erythropoietin- or IFN-treated cells (262). Therefore, JAK-dependent MAP kinase activation by different cytokines or growth factors may occur through recruitment to the JAKs as well as through the well-established pathway involving receptor tyrosine motifs (262). Additional proteins reported to interact with JAKs include SHP1 and SHP2, Vav, Fyn, Btk, Tec, and c-Abl (108, 109, 263–268). For c-Abl, constitutive JAK activation correlated with transformation and was lost on inactivation of a temperature-sensitive c-Abl protein (267). Early suggestive evidence for an additional role for JAKs came from the demonstration that JAK1 is activated in response to EGF but is not required for STAT activation (269). The function of activated JAK1 in this response remains to be established. Similarly, the work of many groups on truncated cytokine receptors has established a requirement for the juxtamembrane Box1 and Box2 motifs and for JAK (but not STAT) activation to stimulate a mitogenic response through pathways yet to be defined (270, 271). IRS1 has been shown to coimmunoprecipitate with

JAK2, and the ability of the growth hormone receptor to transduce the signal for IRS1 depends on the same region of the receptor required for JAK2 binding (272). More recently, work with JAK-null cell lines has established that the activations of IRS1 and phosphatidylinositol 3′ kinase by IL-4, OSM, and IFNs are JAK dependent (87, 273). For IFNs, work with JAK1-null cells has established that JAK1 is required for the activation of cytosolic phospholipase A2 by IFNα (85). Data obtained with a kinase-negative derivative of JAK1 and mutant receptors have raised the possibility that additional JAK-dependent pathways may be required in the antiviral responses to IFNγ and IFNα/β (23, 274). Also, in the most detailed study of the activation of the MAP kinase pathway by IFNα, Larner et al have concluded that JAK1 is essential for the activation of Raf1 and the ERK/MAP kinases (84). Finally, Sugamura et al have implicated JAKs 2 and 3 in activating the signal transducing adaptor molecule, which is involved in both c-myc induction and cell growth in response to IL-2 and GM-CSF (275).

STATs

Evidence is accumulating that STATs play an important role distinct from their well-known function as inducible transcription factors. Three distinct observations reveal an important role for STAT1 in the constitutive expression of certain genes. The expression of IRF1 is low in STAT1-null U3A cells and becomes significant when STAT1 is expressed from a transgene in U3A-R cells (83). The caspase family members ICE, Cpp32, and Ich-1 are expressed at levels 10- to 15-fold lower in U3A cells than in U3A-R or wild-type cells, leading to substantial defects in response to pro-apoptotic signals (275a). Expression of both LMP2 and LMP7 is almost completely absent in U3A cells and is restored in U3A-R cells (Chatterjee-Kishore et al, unpublished data). The defects in caspase expression were corrected when U3A cells were complemented with the Y701F mutant of STAT1, ruling out the possibility that a STAT-STAT dimer stabilized by SH2-phosphotyrosine interactions can be responsible (275a). A strong conclusion is that STAT1 is required for the constitutive expression of some genes, either alone as monomers or, more likely, in combination with transcription-factor partners still to be identified. It remains to be seen whether other STATs have similar functions. As noted above (see also Reference 75), STAT dimers interact with each other and with several different transcription factors, primarily through the N-terminal domains, and STAT2 also uses its N-terminal domain to bind to the IFNAR2c subunit of the receptor (59). We thus imagine that the N-terminal domain of STAT1 may mediate its binding to transcription-factor partners required for constitutive gene expression.

A recent report (49) reveals a scaffolding role for STAT3, which uses its SH2 domain to bind to the tyrosine-phosphorylated cytoplasmic tail of IFNAR1 in the

activated IFNα/β receptor. STAT3 also binds to phosphatidylinositol 3′-kinase, thus bringing it to the receptor. This binding is followed by phosphorylation of phosphatidylinositol 3′-kinase on tyrosine. The functional consequences of this activation by IFNα/β of an additional signaling pathway remain to be elucidated. That at least some of the STATs can serve alternative functions alerts us to the possibility that STAT-null mice may exhibit phenotypes that do not result solely from the lack of STAT activation in response to cytokines or growth factors.

PKR

The activity of PKR in regulating translation is supplemented by its role as a signal-transducing kinase in pathways activated by dsRNA, LPS, and different cytokines (117, 276). In human and mouse cells, activation of PKR by dsRNA leads to activation of NFκB through PKR-mediated phosphorylation of IκB (121, 277–279), and recombinant PKR can activate NFκB and induce DNA-binding activity in cell lysates (277). It is likely that PKR regulates an IκB kinase, and two recent publications have now identified such an enzyme, capable of phosphorylating IκB on the two serine residues appropriate for in vivo function (280, 281).

PKR also plays a role in signal transduction by IFNα, IFNγ, dsRNA, TNFα, LPS, and platelet-derived growth factor (PDGF), revealed largely through experiments with MEFs derived from PKR-null mice. IFNs, dsRNA, TNFα, and LPS all fail to activate the DNA-binding activity of IRF1 in PKR-null MEFs, resulting in a selective defect in the induction of genes dependent on IRF1 (or NFκB) (121, 187, 279). Several genes important in mounting different aspects of host resistance to infection can now be classified as wholly or partially dependent on PKR, including genes involved in antigen presentation (class I MHC), chemotaxis (the chemokines IP-10, myg, JE, and Rantes), antimicrobial activity (iNOS), and apoptosis (FAS). The induction of the cell adhesion molecules VCAM and E-selectin by dsRNA is also mediated through a PKR-dependent pathway (282; S Bandyopadhyay & BRG Williams, unpublished data). Induction of the immunoglobulin κ gene by LPS or IFNγ is mediated by PKR, probably through activation of IRF1 (283).

The mechanisms of activation of PKR by cytokines require further investigation. It is not known whether activation occurs through JAK-dependent pathways or through other signals generated by receptor engagement (for example, Ca^{2+}). An interaction of PKR with STAT1 has been reported (284) but does not appear to be functional, because STAT1-dependent activities are unaffected in PKR-null cells (279). In contrast, the induction of c-fos and c-myc expression by PDGF can be blocked by inhibitors of PKR or by an antisense oligonucleotide against PKR mRNA (285). In accord with this, the

PDGF-induced binding of STAT3 to the GAS element of the c-fos promoter is defective in extracts from PKR-null MEFs compared with extracts from wild-type cells, although the response of STAT3 to other stimuli remains unaffected (A Deb & BRG Williams, unpublished data).

In addition to mediating an important antiviral activity of PKR, the phosphorylation of eIF2α is involved in antiproliferative activities because of this kinase. The most direct evidence comes from studies of the expression of PKR in *Saccharomyces cerevisiae*, where inducible expression of wild-type but not kinase-inactive PKR results in inhibition of growth, which can be reversed by the coexpression of a mutant yeast eIF2α that is not phosphorylated by PKR (286, 287). The induction of tumor formation by mutant PKR proteins (288–290) could be due to failure to appropriately regulate eIF2 or to interactions with other cellular proteins involved in cell growth control.

INVOLVEMENT OF PKR IN APOPTOSIS The mechanisms and signaling mediators that regulate virus-induced apoptosis are not well understood, but it has long been recognized that a combination of IFN and dsRNA is cytotoxic. Because PKR inhibits the growth of yeast and mammalian cells, it is an attractive candidate for involvement in the apoptosis mediated by dsRNA. In support of this idea, overexpression of PKR induces apoptosis through a mechanism dependent on Bcl2 and ICE (291, 292). Normal levels of PKR are required to mediate an apoptotic response to different stimuli, including dsRNA. For example, reduction of PKR levels by antisense oligonucleotides in promonocytic U937 cells inhibits the apoptosis induced by TNFα (293). MEFs derived from PKR-null mice resist apoptotic cell death in response to dsRNA, TNFα, or LPS through a mechanism linked to a defect in activating the DNA-binding activity of IRF1 (187). These results reveal an unexpected role for PKR in mediating stress-induced apoptosis through regulation of IRF1 activity.

Apoptosis is also important in T-cell development. Although thymocytes of wild-type mice express relatively high levels of PKR, the size of the thymus and the ratios of peripheral T-cell subsets are normal in PKR-null mice (S Kadererit & BRG Williams, unpublished data). Therefore, PKR expression in thymocytes is not essential for apoptosis associated with negative and positive selection. Fas mRNA expression is strongly induced in wild-type cells by dsRNA, LPS, and IFNγ, but with the exception of IFNγ, the induction is much reduced in MEFs derived from PKR-null mice (187). Death signals transduced by the Fas receptor depend on the presentation of the ligand Fas L and are largely restricted to a few cell types, such as activated cytotoxic T cells. However, the induction of Fas on wild-type MEFs by dsRNA results in sensitization of the cells to killing by an anti-Fas antibody that stimulates the Fas receptor. MEFs derived from PKR-null mice remain insensitive to killing by this antibody when

treated with dsRNA (S Der & BRG Williams, unpublished data). A role for Fas in virus-induced apoptosis has been suggested for influenza virus (294, 295), and it is likely that PKR is required, although experiments to prove this point remain to be carried out.

RNase L and 2-5A Synthetase

The possible wider role of the 2-5A system in cell metabolism extends beyond the antiviral activity of IFNs. The 2-5A system has been implicated in the action of IFNs. Although RNase L is not essential for normal mouse development (140), the 2-5A system has long been suspected to be involved in RNA decay during cell death. The regression of chick oviducts upon estrogen withdrawal and of rat mammary glands after lactation was correlated with the induction of 2-5A synthetase or with 2-5A per se (296–298). RNase L–null mice have enlarged thymus glands as a result of a defect in apoptosis (140). Thymocytes from these mice were resistant to inducers of apoptosis anti-CD3, anti-fas, staurosporine, and TNFα plus actinomycin D, whereas RNase L–null fibroblasts were resistant to staurosporine or the combination of IFNα and 2-5A. Expression of a dominant negative derivative of RNase L also suppressed apoptosis in cultured cells (J Castelli, BA Hassel, J Paranjape, A Maran, RH Silverman & R Youle, unpublished data). These findings suggest that the control of RNA stability by RNase L plays a role in apoptosis.

An intriguing but unresolved question is whether the 2-5A synthetases do something other than synthesize activators of RNase L. These enzymes differ in structure, intracellular location, activation profiles, and lengths of the 2-5A oligomers produced (127, 130, 299–302). The 2-5A synthetases are versatile enzymes that not only produce 2-5A but also transfer AMP residues in 2′, 5′-linkage to a variety of molecules that terminate in an adenosine residue, such as A5′p35′A, A5′p45′A, NAD, ADP-ribose, and tRNA (303–307). Also, the final nucleotide added by 2-5A synthetase can be something other than AMP (302, 304). Recently, 2-5A synthetases have been used to make pppG2′p5′G by using GTP as a (relatively poor) substrate (302). 2′, 5′-Oligoadenylates with structures different from 2-5A have been observed in cells and tissues of higher vertebrates (298), and some virus-induced alternative 2′, 5′-oligoadenylates can function as inhibitors of RNase L (308). In summary, suggestions of a wider role for the 2-5A synthetases are tantalizing but remain largely unexplored.

IRFs

The IRF family of DNA-binding transcription factors, including IRF1, IRF2, IRF3, ISGF3γ (p48), ICSBP, and ICSAT/PiP/LSIRF, has been implicated in IFN production, cell growth regulation, and induction of gene expression by IFN (100, 309, 310). Experiments using mice null for IRF family members

have recently complemented studies of transfected cell lines and have also provided a link to PKR. IRF1 is essential for mouse *gbp* gene induction by IFNγ (311), and PKR is a signal transducer in this pathway (279). In the absence of PKR, IRF1 DNA binding activity induced in response to IFNγ (or LPS, TNFα, or dsRNA) is deficient. However, certain phenotypes and cellular responses of PKR- and IRF1-null mice are distinct, suggesting both shared and nonoverlapping pathways. For example, IRF1-null mice exhibit reduced levels of $CD8^+$ T cells resulting from a failure of IFNγ to appropriately up-regulate the LMP1 and TAP2 genes, essential for class I MHC function (312–314). Recently it has been shown that IRF1 is required for a TH1 response in vivo (315, 316). PKR-null mice have a normal complement of $CD8^+$ cells in the periphery but exhibit exaggerated contact hypersensitivity, possibly because they fail to induce fas-dependent apoptosis appropriately (121, 187; S Kadereit, R Fairchild & BRG Williams, unpublished data). Both IRF1 and PKR appear to be essential for induction of the inducible nitric oxide synthase gene by IFNγ (317), but unlike IRF1, PKR is not involved in the induction of cell-cycle arrest in response to DNA damage (318; S Der, M Zamanian-Daryouch & BRG Williams, unpublished data). The phenotype of ICSBP-null mice (a lymphoid-specific member of the IRF1 family) is enhanced susceptibility to virus infection as a result of a deficiency in IFNγ production and a chronic myelogenous leukemia-like syndrome that is apparent even in the heterozygotes, suggesting haploin sufficiency (319). Because this phenotype is not shared with the IRF1 or PKR knockouts, ICSBP has a unique role in regulating hematopoiesis.

Literature Cited

1. Darnell JE Jr, Kerr IM, Stark GR. 1994. *Science* 264:1415–21
2. Schindler C, Darnell JE Jr. 1995. *Annu. Rev. Biochem.* 64:621–51
3. Levy DE. 1995. *Virology* 6:181–89
4. Leaman DW, Leung S, Li XX, Stark GR. 1996. *FASEB J.* 10:1578–88
5. Leaman DW. 1998. *Prog. Mol. Subcell. Biol.* 22: In press
6. Ihle JN, Kerr IM. 1995. *Trends Genet.* 11:69–74
7. Ihle JN. 1996. *Cell* 84:331–34
8. Darnell JE Jr. 1996. *Proc. Natl. Acad. Sci. USA* 93:6221–24
9. Darnell JE Jr. 1998. *Science* 277:1630–35
10. Sen GC, Ransohoff RM. 1997. *Transcriptional Regulation in the Interferon System.* Georgetown, TX: Landes Bio-Sci.
11. Bach EA, Aguet M, Schreiber RD. 1997. *Annu. Rev. Immunol.* 15:563–91
12. Farrar MA, Schreiber RD. 1993. *Annu. Rev. Immunol.* 11:571–611
13. Soh J, Donnelly RJ, Kotenko S, Mariano TM, Cook JR, et al. 1994. *Cell* 76:793–802
14. Hemmi S, Bohni R, Stark G, DiMarco F, Aguet M. 1994. *Cell* 76:803–10
15. Bach EA, Tanner JW, Marsters SA, Ashkenazi A, Aguet M, et al. 1996. *Mol. Cell. Biol.* 16:3214–21
16. Kotenko SV, Izotova LS, Pollack BP, Mariano TM, Donnelly RJ, et al. 1995. *J. Biol. Chem.* 270:20915–21
17. Sakatsume M, Igarashi K, Winestock

KD, Garotta G, Larner AC, Finbloom DS. 1995. *J. Biol. Chem.* 270:17528–34
18. Kaplan DH, Greenlund AC, Tanner JW, Shaw AS, Schreiber RD. 1996. *J. Biol. Chem.* 271:9–12
19. Fountoulakis M, Zulauf M, Lustig A, Garotta G. 1992. *Eur. J. Biochem.* 208: 781–87
20. Greenlund AC, Schreiber RD, Goeddel DV, Pennica D. 1993. *J. Biol. Chem.* 268:18103–10
21. Walter MR, Windsor WT, Nagabhushan TL, Lundell DJ, Lunn CA, et al. 1995. *Nature* 376:230–35
22. Marsters S, Pennica D, Bach E, Schreiber RD, Ashkenazi A. 1995. *Proc. Natl. Acad. Sci. USA* 92:5401–5
23. Briscoe J, Rogers NC, Witthuhn BA, Watling D, Harpur AG, et al. 1996. *EMBO J.* 15:799–809
24. Kotenko SV, Izotova LS, Pollack BP, Muthukumaran G, Paukku K, et al. 1996. *J. Biol. Chem.* 271:17174–82
25. Kohlhuber F, Rogers NC, Watling D, Feng J, Guschin D, et al. 1997. *Mol. Cell. Biol.* 17:695–706
26. Greenlund AC, Farrar MA, Viviano BL, Schreiber RD. 1994. *EMBO J.* 13:1591–600
27. Igarashi K, Garotta G, Ozmen L, Ziemieckl A, Wilks AF, et al. 1994. *J. Biol. Chem.* 269:14333–36
28. Greenlund AC, Morales MO, Viviano BL, Yan H, Krolewski J, Schreiber RD. 1995. *Immunity* 2:677–87
29. Heim MH, Kerr IM, Stark GR, Darnell JE Jr. 1995. *Science* 267:1347–49
30. Schindler C, Shuai K, Prezioso VR, Darnell JE Jr. 1992. *Science* 257:809–13
31. Shuai K, Schindler C, Prezioso VR, Darnell JE Jr. 1992. *Science* 258:1808–12
32. Shuai K, Stark GR, Kerr IM, Darnell JE Jr. 1993. *Science* 261:1744–46
33. Sekimoto T, Nakajima K, Tachibana T, Hirano T, Yoneda Y. 1997. *J. Biol. Chem.* 271:31017–20
34. Wen ZL, Zhong Z, Darnell JE Jr. 1995. *Cell* 82:241–50
35. David M, Petricoin E III, Benjamin C, Pine R, Weber MJ, Larner AC. 1995. *Science* 269:1721–23
36. Pernis A, Gupta S, Gollob KJ, Garfein E, Coffman RL, et al. 1995. *Science* 269:245–47
37. Bach EA, Szabo SJ, Dighe AS, Ashkenazi A, Aguet M, et al. 1995. *Science* 270:1215–18
38. Starr R, Willson TA, Viney EM, Murray LJL, Rayner JR, et al. 1997. *Nature* 387:917–21
39. Endo TA, Masuhara M, Yokouchi M, Suzuki R, Sakamoto H, et al. 1997. *Nature* 387:921–24
40. Naka T, Narazaki M, Hirata M, Matsumoto T, Minamoto S, et al. 1997. *Nature* 387:924–29
41. Meraz MA, White JM, Sheehan KCF, Bach EA, Rodig SJ, et al. 1996. *Cell* 84:431–42
42. Durbin JE, Hackenmiller R, Simon MC, Levy DE. 1996. *Cell* 84:443–50
43. Reid LE, Brasnett AH, Gilbert CS, Porter ACG, Gewert DR, et al. 1997. *Proc. Natl. Acad. Sci. USA* 86:840–44
44. Bluyssen HAR, Muzaffar R, Vlieststra RJ, van der Made ACJ, Leung S, et al. 1995. *Proc. Natl. Acad. Sci. USA* 92: 5645–49
45. Look DC, Pelletier MR, Tidwell RM, Roswit WT, Holtzman MJ. 1997. *J. Biol. Chem.* 270:30264–67
46. Perez C, Wietzerbin J, Benech PD. 1997. *Mol. Cell. Biol.* 13:2182–92
47. Perez C, Coeffier E, Moreau-Gachelin F, Wietzerbin J, Benech PD. 1997. *Mol. Cell. Biol.* 14:5023–31
48. Novick D, Cohen B, Rubinstein M. 1994. *Cell* 77:391–400
49. Pfeffer LM, Basu L, Pfeffer SR, Yang CH, Murti A, et al. 1997. *J. Biol. Chem.* 272:11002–5
50. Lutfalla G, Holland SJ, Cinato E, Monneron D, Reboul J, et al. 1995. *EMBO J.* 14:5100–8
51. Russell-Harde D, Pu HF, Betts M, Harkins RN, Perez HD, Croze E. 1995. *J. Biol. Chem.* 270:26033–36
52. Cohen B, Novick D, Barak S, Rubinstein M. 1995. *Mol. Cell. Biol.* 15:4208–14
53. Colamonici O, Yan H, Domanski P, Handa R, Smalley D, et al. 1994. *Mol. Cell. Biol.* 14:8133–42
54. Colamonici OR, Uyttendaele H, Domanski P, Yan H, Krolewski JJ. 1994. *J. Biol. Chem.* 269:3518–22
55. Gauzzi MC, Velazquez L, McKendry R, Mogensen KE, Fellous M, Pellegrini S. 1996. *J. Biol. Chem.* 271:20494–500
56. Pellegrini S, John J, Shearer M, Kerr IM, Stark GR. 1989. *Mol. Cell. Biol.* 9:4605–12
57. Velazquez L, Mogensen KE, Barbieri G, Fellous M, Uzé G, Pellegrini S. 1995. *J. Biol. Chem.* 270:3327–34
58. Krishnan K, Yan H, Lim JTE, Krolewski JJ. 1996. *Oncogene* 13:125–33
59. Li XX, Leung S, Kerr IM, Stark GR. 1997. *Mol. Cell. Biol.* 17:2048–56
60. Stancato LF, David M, Carter-Su C, Larner AC, Pratt WB. 1996. *J. Biol. Chem.* 271:4134–37

61. Yan H, Krishnan K, Greenlund AC, Gupta S, Lim JTE, et al. 1996. *EMBO J.* 15:1064–74
62. David M, Zhou GC, Pine R, Dixon JE, Larner AC. 1996. *J. Biol. Chem.* 271: 15862–65
63. John J, McKendry R, Pellegrini S, Flavell D, Kerr IM, Stark GR. 1991. *Mol. Cell. Biol.* 11:4189–95
64. Bluyssen HAR, Muzaffar R, Vlieststra RJ, van der Made ACJ, Leung S, et al. 1995. *Proc. Natl. Acad. Sci. USA* 92: 5645–49
65. Harada H, Matsumoto M, Sato M, Kashiwazaki Y, Kimura T, et al. 1996. *Genes Cells* 1:995–1005
66. Haque SJ, Williams BRG. 1994. *J. Biol. Chem.* 269:19523–29
67. Li XX, Leung S, Qureshi S, Darnell JE Jr, Stark GR. 1996. *J. Biol. Chem.* 271:5790–94
68. Dale TC, Rosen JM, Guille MJ, Lewin AR, Porter AGC, et al. 1989. *EMBO J.* 8:831–39
69. Imam AMA, Ackrill AM, Dale TC, Kerr IM, Stark GR. 1990. *Nucleic Acids Res.* 18:6573–80
70. Qureshi SA, Salditt-Georgieff M, Darnell JE Jr. 1995. *Proc. Natl. Acad. Sci. USA* 92:3829–33
71. Bluyssen HAR, Levy DE. 1997. *J. Biol. Chem.* 272:4600–5
72. Horvath CM, Wen ZL, Darnell JE Jr. 1995. *Genes Dev.* 9:984–94
73. Horvath CM, Stark GR, Kerr IM, Darnell JE Jr. 1996. *Mol. Cell. Biol.* 16: 6957–64
74. Decker T, Kovarik P, Meinke A. 1997. *J. Interferon Cytokine Res.* 17:121–34
75. Xu XA, Sun Y-L, Hoey T. 1996. *Science* 273:794–97
76. Vinkemeier U, Cohen SL, Moarefi I, Chait BT, Kuriyan J, Darnell JE Jr. 1996. *EMBO J.* 15:5616–26
77. Guyer NB, Severns CW, Wong P, Feghali CA, Wright TM. 1995. *J. Immunol.* 155:3472–80
78. Porter ACG, Chernajovsky Y, Dale TC, Gilbert CS, Stark GR, Kerr IM. 1988. *EMBO J.* 7:85–92
79. Zhang JJ, Vinkemeier U, Gu W, Chakravarti D, Horvath CM, Darnell JE Jr. 1996. *Proc. Natl. Acad. Sci. USA* 93: 15092–96
80. Horvai AE, Xu L, Korzus E, Brard G, Kalafus D, et al. 1997. *Proc. Natl. Acad. Sci. USA* 94:1074–79
81. Rutherford MN, Kumar A, Haque SJ, Ghysdael J, Williams BRG. 1997. *J. Interferon Cytokine Res.* 17:1–10
82. Bhattacharya S, Eckner R, Grossman S, Oldread E, Arany Z, et al. 1996. *Nature* 383:344–47
83. Müller M, Laxton C, Briscoe J, Schindler C, Improta T, et al. 1993. *EMBO J.* 12:4221–28
84. Stancato LF, Sakatsume M, David M, Dent P, Dong F, et al. 1997. *Mol. Cell. Biol.* 17:3833–40
85. Flati V, Haque SJ, Williams BRG. 1996. *EMBO J.* 15:1566–71
86. Uddin S, Yenush L, Sun X-J, Sweet ME, White MF, Platanias LC. 1995. *J. Biol. Chem.* 270:15938–41
87. Burfoot MS, Rogers NC, Watling D, Smith JM, Pons S, et al. 1997. *J. Biol. Chem.* 272:24183–90
88. Diaz M, Bohlander S, Allen G. 1993. *J. Interferon Res.* 13:243–44
89. Weck P, Apperson S, May L, Stebbing N. 1981. *J. Gen. Virol.* 57:233–37
90. Evinger M, Rubinstein M, Pestka S. 1981. *Arch. Biochim. Biophys.* 210:319–29
91. Foster GR, Rodrigues O, Ghouze F, Schulte-Frohlinde E, Testa D, et al. 1996. *J. Interferon Cytokine Res.* 16: 1027–33
92. Pestka S, Langer JA, Zoon KC, Samuel CE. 1987. *Annu. Rev. Biochem.* 56:727–77
93. Platanias LC, Uddin S, Colamonici OR. 1994. *J. Biol. Chem.* 269:17761–64
94. Abramovich C, Shulman LM, Ratovitski E, Harroch S, Tovey M, et al. 1994. *EMBO J.* 13:5871–77
95. Platanias LC, Uddin S, Domanski P, Colamonici OR. 1996. *J. Biol. Chem.* 271:23630–33
96. Croze E, Russell-Harde D, Wagner TC, Pu HF, Pfeffer LM, Perez HD. 1996. *J. Biol. Chem.* 271:33165–68
97. Rani MRS, Foster GR, Leung S, Leaman D, Stark GR, Ransohoff RM. 1996. *J. Biol. Chem.* 271:22878–84
98. Harada H, Fujita T, Miyamoto M, Kimurak Y, Murayama M, et al. 1989. *Cell* 58:729–39
99. Nelson N, Marks MS, Driggers PH, Ozato K. 1993. *Mol. Cell. Biol.* 13:588–99
100. Yamagata T, Nishida J, Tanaka S, Sakai R, Mitani K, et al. 1996. *Mol. Cell. Biol.* 16:1283–94
101. Friedman RL, Manly SP, McMahon M, Kerr IM, Stark GR. 1984. *Cell* 38:745–55
102. Larner AC, Chaudhuri A, Darnell JE Jr. 1986. *J. Biol. Chem.* 261:453–59
103. David M, Grimley PM, Finbloom DS, Larner AC. 1993. *Mol. Cell. Biol.* 13: 7515–21

104. Haspel RL, Salditt-Georgieff M, Darnell JE Jr. 1996. *EMBO J.* 15:6262–68
105. Kim TK, Maniatis T. 1996. *Science* 273: 1717–19
106. Igarashi K-I, David M, Larner AC, Finbloom DS. 1993. *Mol. Cell. Biol.* 13: 3984–89
107. Haque SJ, Flati V, Deb A, Williams BRG. 1995. *J. Biol. Chem.* 270:25709–14
108. David M, Chen HE, Goelz S, Larner AC, Neel BG. 1995. *Mol. Cell. Biol.* 15:7050–58
109. Jiao HY, Berrada K, Yang WT, Tabrizi M, Platanias LC, Yi TL. 1996. *Mol. Cell. Biol.* 16:6985–92
110. Petricoin EF III, David M, Igarashi K, Benjamin C, Ling L, et al. 1996. *Mol. Cell. Biol.* 16:1419–24
111. Petricoin EF III, Hackett RH, Akai H, Igarashi K, Finbloom DS, Larner AC. 1992. *Mol. Cell. Biol.* 12:4486–95
112. Kimura T, Kadokawa Y, Harada H, Matsumoto M, Sato M, et al. 1996. *Genes Cells* 1:115–24
113. Isaacs A, Lindenmann J. 1957. *Proc. R. Soc. London Ser. B* 147:258–67
114. van den Broek MF, Muller U, Huang S, Aguet M, Zinkernagel RM. 1995. *J. Virol.* 69:4792–96
115. Vilcek J, Sen GC. 1996. In *Fields Virology*, ed. BN Fields, DM Knipe, PM Howley, pp. 375–400. Philadelphia: Lippincott-Raven. 3rd ed.
116. Meurs E, Chong K, Galabru J, Thomas NS, Kerr IM, et al. 1990. *Cell* 62:379–90
117. McMillan NAJ, Williams BRG. 1996. In *Protein Phosphorylation in Cell Growth Regulation*, ed. MJ Clemens, pp. 225–54. London: Harwood Acad.
118. Carpick BW, Graziano V, Schneider D, Maitra RK, Lee X, Williams BRG. 1997. *J. Biol. Chem.* 272:9510–16
119. Meurs EF, Watanabe Y, Kadereit S, Barber GN, Katze MG, et al. 1992. *J. Virol.* 66:5805–14
120. Der SD, Lau AS. 1995. *Proc. Natl. Acad. Sci. USA* 92:8841–45
121. Yang YL, Reis LFL, Pavlovic J, Aguzzi A, Schafer R, et al. 1995. *EMBO J.* 14:6095–106
122. Kerr IM, Brown RE. 1978. *Proc. Natl. Acad. Sci. USA* 75:256–60
123. Silverman RH, Cirino NM. 1997. In *mRNA Metabolism and Post-Transcriptional Gene Regulation*, ed. DR Morris, JB Harford, pp. 295–309. New York: Wiley & Sons
124. Wreschner DH, McCauley JW, Skehel JJ, Kerr IM. 1981. *Nature* 289:414–17
125. Floyd-Smith G, Slattery E, Lengyel P. 1981. *Science* 212:1030–32
126. Carroll SS, Chen E, Viscount T, Geib J, Sardana MK, et al. 1996. *J. Biol. Chem.* 271:4988–92
127. Chebath J, Benech P, Hovanessian AG, Galabru J, Revel M. 1987. *J. Biol. Chem.* 262:3852–57
128. Ghosh SK, Kusari J, Bandyopadhyay SK, Samanta H, Kumar R, Sen GC. 1991. *J. Biol. Chem.* 266:15293–99
129. Rutherford MN, Kumar A, Nissim A, Chebath J, Williams BRG. 1991. *Nucleic Acids Res.* 19:1917–24
130. Marie I, Hovanessian AG. 1992. *J. Biol. Chem.* 267:9933–39
131. Dong BH, Silverman RH. 1995. *J. Biol. Chem.* 270:4133–37
132. Cole JL, Carroll SS, Kuo LC. 1996. *J. Biol. Chem.* 271:3979–81
133. Cole JL, Carroll SS, Blue ES, Viscount T, Kuo LC. 1997. *J. Biol. Chem.* 272: 19187–92
134. Carroll SS, Cole JL, Viscount T, Geib J, Gehman J, Kuo L. 1997. *J. Biol. Chem.* 272:19193–98
135. Zhou AM, Hassel BA, Silverman RH. 1993. *Cell* 72:753–65
136. Hassel BA, Zhou AM, Sotomayor C, Maran A, Silverman RH. 1993. *EMBO J.* 12:3297–304
137. Dong B, Silverman RH. 1997. *J. Biol. Chem.* 272:22236–42
138. Sidrauski C, Walter P. 1997. *Cell* 90: 1031–39
139. Diaz-Guerra M, Rivas C, Esteban M. 1997. *Virology* 227:220–28
140. Zhou A, Paranjape J, Brown TL, Nie H, Naik S, et al. 1997. *EMBO J.* 16:6355–63
141. Torrence PF, Maitra RK, Lesiak K, Khamnei S, Zhou A, Silverman RH. 1993. *Proc. Natl. Acad. Sci. USA* 90: 1300–4
142. Cirino NM, Li GY, Xiao W, Torrence PF, Silverman RH. 1997. *Proc. Natl. Acad. Sci. USA* 94:1937–42
143. Mitra A, Higgins DW, Langenberg WG, Nie HQ, Sengupta DN, Silverman RH. 1996. *Proc. Natl. Acad. Sci. USA* 93:6780–85
144. Ogawa T, Hori T, Ishida I. 1996. *Nat. Biotechnol.* 14:1566–69
145. Horisberger MA. 1995. *Am. J. Respir. Crit. Care Med.* 152:S67-S71
146. Arnheiter H, Frese M, Kambadur R, Meier E, Haller O. 1996. *Curr. Top. Microbiol. Immunol.* 206:119–47
147. Takei K, McPherson PS, Schmid SL, De Camilli P. 1995. *Nature* 374:186–90

148. Hinshaw JE, Schmid SL. 1995. *Nature* 374:190–92
149. Nakayama M, Yazaki K, Kusano A, Nagata K, Hanai N, Ishihama A. 1993. *J. Biol. Chem.* 268:15033–38
150. Richter MF, Schwemmle M, Herrmann C, Wittinghofer A, Staeheli P. 1995. *J. Biol. Chem.* 270:13512–17
151. Ponten A, Sick C, Weeber M, Haller O, Kochs G. 1997. *J. Virol.* 71:2591–99
152. Haller O, Frese M, Rost D, Nuttal PA, Kochs G. 1995. *Virology* 4:2596–601
153. Schnorr J-J, Schneider-Schaulies S, Simon-Jodicke A, Pavlovic J, Horisberger MA, ter Meulen V. 1993. *J. Virol.* 67:4760–68
154. Thimme R, Frese M, Kochs G, Haller O. 1995. *Virology* 211:296–301
155. Frese M, Kochs G, Feldmann H, Hertkorn C, Haller O. 1996. *J. Virol.* 70: 915–23
156. Zhao H, De BP, Das T, Banerjee AK. 1996. *Virology* 220:330–38
157. Frese M, Kochs G, Meier-Dieter U, Siebler J, Haller O. 1995. *J. Virol.* 69:3904–9
158. Pitossi F, Blank A, Schroder A, Schwarz A, Hussi P, Schwemmle M. 1993. *J. Virol.* 67:6726–32
159. Schwemmle M, Weining KC, Richter MF, Schumacher B, Staeheli P. 1995. *Virology* 206:545–54
160. Stranden AM, Staeheli P, Pavlovic J. 1993. *Virology* 197:642–51
161. Pavlovic J, Haller O, Staeheli P. 1992. *J. Virol.* 66:2564–69
162. Floyd-Smith GJ. 1988. *Cell. Biochem.* 38:13–21
163. Staeheli P, Horisberger MA, Haller O. 1984. *Virology* 132:456–61
164. Karupiah G, Xie QW, Buller RM, Nathan C, Duarte C, MacMicking JD. 1993. *Science* 261:1445–48
165. Alber D, Staeheli P. 1996. *J. Interferon Cytokine Res.* 16:375–80
166. Basinga M. 1992. *Science* 258:1730–31
167. Gooding LR. 1992. *Cell* 71:5–7
168. McFadden G, Graham K, Ellison K, Barry M, Macen J, et al. 1995. *J. Leukocyte Biol.* 57:731–38
169. Moore PS, Boshoff C, Weiss RA, Chang Y. 1996. *Science* 274:1739–44
170. Smith GL. 1996. *Curr. Opin. Immunol.* 8:467–71
171. Cayley PJ, Knight M, Kerr IM. 1982. *Biochem. Biophys. Res. Commun.* 104: 376–82
172. Bisbal C, Martinand C, Silhol M, Lebleu B, Salehzada T. 1995. *J. Biol. Chem.* 270:13308–17
173. Kitajewski J, Schneider RJ, Safer B, Munemitsu SM, Samuel CE, et al. 1986. *Cell* 45:195–200
174. Anderson KP, Fennie EH. 1987. *J. Virol.* 61:787–95
175. Clarke PA, Schwemmle M, Schikinger J, Hilse K, Clemens M. 1991. *Nucleic Acids Res.* 19:243–48
176. Swaminathan S, Honeycut BS, Reiss CS, Kieff E. 1992. *J. Virol.* 66:5133–36
177. Imani F, Jacobs BL. 1988. *Proc. Natl. Acad. Sci. USA* 85:7887–91
178. Beattie E, Denzler KL, Tartaglia J, Perkus ME, Paoletti E, Jacobs BL. 1995. *J. Virol.* 69:499–505
179. Roy S, Katze MG, Parkin NT, Edery I, Hovanessian AG, Sonenberg N. 1990. *Science* 247:1216–20
180. McMillan NA, Chun RF, Siderovski DP, Galabru J, Toone WM, et al. 1995. *Virology* 213:413–24
181. Gale MJ Jr, Korth MJ, Tang NM, Tan SL, Hopkins DA, et al. 1997. *Virology* 230:217–27
182. Black TL, Safer B, Hovanessian AG, Katze MG. 1989. *J. Virol.* 63:2244–51
183. Dubois MF, Hovanessian AG. 1990. *Virology* 179:591–98
184. Melville MW, Hansen WJ, Freeman BC, Welch WJ, Katze MG. 1997. *Proc. Natl. Acad. Sci. USA* 94:97–102
185. Swaminathan S, Rajan P, Savinova O, Jagus R, Thimmapaya B. 1996. *Virology* 219:321–23
186. Shen Y, Shenk TE. 1995. *Curr. Biol.* 5:105–11
187. Der SD, Yang Y-L, Weissmann C, Williams BRG. 1997. *Proc. Natl. Acad. Sci. USA* 94:3279–83
188. Ray CA, Black RA, Kronheim SR, Greenstreet TA, Sleath PR, et al. 1992. *Cell* 69:597–604
189. Thome M, Schneider P, Hofmann K, Fickenscher H, Meinl E, et al. 1997. *Nature* 386:517–21
190. Henderson S, Huen D, Rowe M, Dawson C, Johnson G, Rickinson A. 1993. *Proc. Natl. Acad. Sci. USA* 90:8479–83
191. Reich N, Pine R, Levy D, Darnell JE. 1988. *J. Virol.* 62:114–19
192. Ackrill AM, Foster GR, Laxton CD, Flavell DM, Stark GR, Kerr IM. 1991. *Nucleic Acids Res.* 19:4387–93
193. Leonard GT, Sen GC. 1997. *J. Virol.* 71:5095–101
194. Perea SE, Lopez-Ocejo O, Von Gabain A, Arana MDJ. 1997. *Int. J. Oncol.* 11: 169–73
195. Symons JA, Alcami A, Smith GL. 1995. *Cell* 81:551–60

196. Stuart AD, Stewart AP, Arrand JR, MacKett M. 1995. *Oncogene* 11:1711–19
197. Deiss LP, Feinstein E, Berissi H, Cohen O, Kimchi A. 1995. *Genes Dev.* 9:15–30
198. Higuchi T, Hannigan GE, Malkin D, Yeger H, Williams BRG. 1991. *Cancer Res.* 51:3958–64
199. Nason-Burchenal K, Gandini D, Bott M, Allpenna J, Seale JR, Cross NC, et al. 1996. *Blood* 88:3926–36
200. Kumar R, Atlas I. 1992. *Proc. Natl. Acad. Sci. USA* 89:6599–603
201. Resnitzky D, Tiefenbrun N, Berissi H, Kimchi A. 1992. *Proc. Natl. Acad. Sci. USA* 89:402–6
202. Melamed D, Tiefenbrun N, Yarden A, Kimchi A. 1993. *Mol. Cell. Biol.* 12: 5255–65
203. Tiefenbrun N, Melamed D, Levy N, Resnitzky D, Hoffmann I, et al. 1996. *Mol. Cell. Biol.* 16:3934–44
204. Raveh T, Hovanessian AG, Meurs EF, Sonenberg N, Kimchi A. 1996. *J. Biol. Chem.* 271:25479–84
205. Deng C, Zhang P, Harper JW, Elledge SJ, Leder P. 1995. *Cell* 82:675–84
206. Hobeika AC, Subramaniam P, Johnson HM. 1997. *Oncogene* 14:1165–70
207. Grawunder U, Melchers F, Rolink A. 1993. *Eur. J. Immunol.* 23:544–51
208. Rojas R, Roman J, Torres A, Ramirez R, Carracedo J. 1996. *Leukemia* 10:1782–88
209. Buschle M, Campana D, Carding SR, Richard C, Hoffbrand AV, Breener MK. 1993. *J. Exp. Med.* 177:213–18
210. Novelli F, Di Pierro F, di Celle PF, Bertini S, Affaticati P, et al. 1994. *J. Immunol.* 152:496–504
211. Ghayur T, Banerjee S, Hugunin M, Butler D, Herzog L, et al. 1997. *Nature* 386:619–23
212. Gu Y, Kuida K, Tsutsui H, Ku G, Hsiao K, et al. 1997. *Science* 275:206–9
213. Deiss LP, Kimchi A. 1991. *Science* 252:117–20
214. Kissil JL, Deiss LP, Bayewitch M, Raveh T, Khaspekov G, Kimchi A. 1995. *J. Biol. Chem.* 270:27932–36
215. Feinstein E, Kimchi A, Wallach D, Boldin M, Varfolomeev E. 1995. *Trends Biochem. Sci.* 20:342–44
216. Deiss LP, Galinka H, Berissi H, Cohen O, Kimchi A. 1996. *EMBO J.* 15:3861–70
217. Levy-Strumpf N, Deiss LP, Berissi H, Kimchi A. 1997. *Mol. Cell. Biol.* 17: 1615–25
218. Cohen O, Feinstein E, Kimchi A. 1997. *EMBO J.* 16:998–1008
219. Kissil JL, Feinstein E, Cohen O, Jones PA, Tsai YC, et al. 1997. *Oncogene* 15:403–7
219a. Inbal B, Cohen O, Polak-Charcon S, Kopolovic J, Vadai E, et al. 1997. *Nature* 390:180–84
220. Uzé G, Lutfalla G, Mogensen KE. 1995. *J. Interferon Res.* 15:3–26
221. Boehm U, Klamp T, Groot M, Howard JC. 1997. *Annu. Rev. Immunol.* 15:749–95
222. Reis LFL, Harada H, Wolchok JD, Taniguchi T, Vilcek J. 1992. *EMBO J.* 11:185–93
223. Chang C-H, Hammer J, Loh JE, Fodor WL, Flavell RA. 1992. *Immunogenetics* 35:378–84
224. Mach B, Steimle V, Martinez-Soria E, Reith W. 1996. *Annu. Rev. Immunol.* 14:301–31
225. Mond JJ, Carman J, Sarma C, Ohara J, Finkelman FD. 1986. *J. Immunol.* 137:3534–37
226. York IA, Rock KL. 1996. *Annu. Rev. Immunol.* 14:369–96
227. Boes B, Hengel H, Ruppert T, Multhaup G, Koszinowski UH, Kloetzel PM. 1994. *J. Exp. Med.* 179:901–9
228. Groettrup M, Soza A, Eggers M, Keuhn L, Dick TP, et al. 1996. *Nature* 381:166–68
229. Trowsdale J, Hanson I, Mockridge I, Beck S, Townsend A, Kelly A. 1990. *Nature* 348:741–44
230. Epperson DE, Arnold E, Spies T, Creswell P, Pober JS, Johnson DR. 1992. *J. Immunol.* 149:3297–301
231. Abbas AK, Murphy KM, Sher A. 1997. *Nature* 383:787–93
232. Hsieh C-S, Macatonia S, Tripp CS, Wolf SF, O'Garra A, Murphy KM. 1993. *Science* 260:547–49
233. Murphy TL, Cleveland MG, Kulesza P, Magram J, Murphy KM. 1995. *Mol. Cell. Biol.* 15:5258–67
234. Dighe AS, Campbell D, Hsieh C-S, Clarke S, Greaves DR, et al. 1995. *Immunity* 3:657–66
235. Flesch IEA, Hess JH, Huang S, Aguet M, Rothe J, et al. 1995. *J. Exp. Med.* 181:1615–21
236. Trinchieri G. 1995. *Annu. Rev. Immunol.* 13:251–76
237. Szabo SJ, Dighe AS, Gubler U, Murphy KM. 1997. *J. Exp. Med.* 185:817–24
238. Szabo SJ, Jacobson NG, Dighe AS, Gubler U, Murphy KM. 1995. *Immunity* 2:665–75
239. Gajewski TF, Fitch FW. 1988. *J. Immunol.* 140:4245–52
240. Adams DO, Hamilton TA. 1984. *Annu. Rev. Immunol.* 2:283–318

241. Buchmeier NA, Schreiber RD. 1985. *Proc. Natl. Acad. Sci. USA* 82:7404–8
242. Dalton DK, Pitts-Meek S, Keshav S, Figari IS, Bradley A, Stewart TA. 1993. *Science* 259:1739–42
243. Huang S, Hendriks W, Althage A, Hemmi S, Bluethmann H, et al. 1993. *Science* 259:1742–45
244. Newport MJ, Huxley CM, Huston S, Hawrylowicz CM, Oostra BA, et al. 1996. *N. Engl. J. Med.* 335:1941–49
245. Jouanguy E, Altare F, Lamhamedi S, Revy P, Newport M, et al. 1996. *N. Engl. J. Med.* 335:1956–61
246. Klebanoff SJ. 1992. In *Inflammation: Basic Principles and Clinical Correlates*, ed. JI Gallin, pp. 541–89. New York: Raven. 2nd ed.
247. MacMicking J, Xie Q-W, Nathan C. 1997. *Annu. Rev. Immunol.* 15:323–50
248. Snapper CM, Peschel C, Paul WE. 1988. *J. Immunol.* 140:2121–27
249. Snapper CM, McIntyre TM, Mandler R, Pecanha LMT, Finkelman FD, et al. 1992. *J. Exp. Med.* 175:1367–71
250. Snapper CM, Paul WE. 1987. *Science* 236:944–47
251. Finkelman FD, Svetic A, Gresser I, Snapper C, Holmes J, et al. 1991. *J. Exp. Med.* 174:1179–88
252. Wang Y-D, Wong K, Wood WI. 1995. *J. Biol. Chem.* 270:7021–24
253. Fujitani Y, Hibi M, Fukada T, Takahashi-Tezuka M, Yoshida H, et al. 1997. *Oncogene* 14:751–61
254. Lobie PE, Ronsin B, Silvennoinen O, Haldosen LA, Norstedt G, Morel G. 1996. *Endocrinology* 137:4037–45
255. Conway G, Margoliath A, Wongmadden S, Roberts RJ, Gilbert W. 1997. *Proc. Natl. Acad. Sci. USA* 94:3082–87
256. Russell SM, Tayebi N, Nakajima H, Riedy MC, Roberts JL, et al. 1995. *Science* 270:797–800
257. Nosaka T, van Deursen JM, Tripp RA, Thierfelder WE, Witthuhn BA, et al. 1995. *Science* 270:800–2
258. Smit LS, Meyer DJ, Billestrup N, Norstedt G, Schwartz J, Carter-Su C. 1996. *Mol. Endocrinol.* 10:519–33
259. Wang XY, Fuhrer DK, Feng GS, Marshall MS, Yang Y-C. 1995. *Blood* 86: 608
260. Chauhan D, Kharbanda SM, Ogata A, Urashima M, Frank D, et al. 1995. *J. Exp. Med.* 182:1801–6
261. Han YL, Leaman DW, Watling D, Rogers NC, Groner B, et al. 1996. *J. Biol. Chem.* 271:5947–52
262. Winston LA, Hunter T. 1996. *Curr. Biol.* 6:668–71
263. Yin TG, Shen R, Feng GS, Yang YC. 1997. *J. Biol. Chem.* 272:1032–37
264. Uddin S, Sweet M, Colamonici OR, Krolewski JJ, Platanias LC. 1997. *FEBS Lett.* 403:31–34
265. Uddin S, Sher DA, Alsayed Y, Pons S, Colamonici OR, et al. 1997. *Biochem. Biophys. Res. Commun.* 235:83–88
266. Takahashitezuka M. 1997. *Oncogene* 14:2273–82
267. Danial NN, Pernis A, Rothman PB. 1995. *Science* 269:1875–77
268. Uddin S, Gardziola C, Dangat A, Yi T, Platanias LC. 1996. *Biochem. Biophys. Res. Commun.* 225:833–38
269. Leaman DW, Pisharody S, Flickinger TW, Commane MA, Schlessinger J, et al. 1996. *Mol. Cell. Biol.* 16:369–75
270. Hirano T. 1998. *Int. Rev. Immunol.* 16: 249–84
271. Kishimoto T, Akira S, Narazaki M, Taga T. 1995. *Blood* 86:1243–54
272. Argetsinger LS, Hsu GW, Myers MG Jr, Billestrup N, White MF, Carter-Su C. 1995. *J. Biol. Chem.* 270:14685–92
273. Wang HY, Zamorano J, Yoerkie JL, Paul WE, Keegan AD. 1997. *J. Immunol.* 158:1037–40
274. Novick D, Cohen B, Kim HS, Levy Y, Rubinstein M. 1996. *Eur. Cytokine Netw.* 7:523
275. Takeshita T, Arita T, Higuchi M, Asao H, Endo K, et al. 1997. *Immunity* 6:449–57
275a. Kumar A, Commane M, Flickinger TW, Horvath CM, Stark GR. 1997. *Science* 278:1630–32
276. Williams BRG. 1995. *Semin. Virol.* 6: 191–202
277. Kumar A, Haque J, Lacoste J, Hiscott J, Williams BR. 1994. *Proc. Natl. Acad. Sci. USA* 91:6288–92
278. Maran A, Maitra RK, Kumar A, Dong BH, Xiao W, et al. 1994. *Science* 265: 789–92
279. Kumar A, Yang Y-L, Flati V, Der S, Kadereit S, et al. 1997. *EMBO J.* 16:406–16
280. DiDonato JA, Hayakawa M, Rothwarf DM, Zandi E, Karin M. 1997. *Nature* 388:548–53
281. Regnier CH, Song HY, Gao X, Goeddel DV, Cao Z, Rothe M. 1997. *Cell* 90:373–83
282. Offerman MK, Simring J, Mellits KH, Hagan MK, Shaw R, et al. 1995. *Eur. J. Biochem.* 232:28–36
283. Koromilas AE, Cantin C, Craig AWB, Jagus R, Hiscott J, Sonenberg N. 1995. *J. Biol. Chem.* 270:25426–34

284. Wong AH-T, Tam NWN, Yang Y-L, Cuddihy AR, Li S, et al. 1997. *EMBO J.* 16:1291–1304
285. Mundschau LJ, Faller DV. 1995. *J. Biol. Chem.* 270:3100–6
286. Chong KL, Feng L, Schappert K, Meurs E, Donahue TF, et al. 1992. *EMBO J.* 11:1553–62
287. Dever TE, Chen J-J, Barber GN, Cigan AM, Feng L, et al. 1993. *Proc. Natl. Acad. Sci. USA* 90:4616–20
288. Koromilas AE, Roy S, Barber GN, Katze MG, Sonenberg N. 1992. *Science* 257:1685–89
289. Meurs EF, Galabru J, Barber GN, Katze MG, Hovanessian AG. 1993. *Proc. Natl. Acad. Sci. USA* 90:232–36
290. Barber GN, Wambach M, Thompson S, Jagus R, Katze MG. 1995. *Mol. Cell. Biol.* 15:3138–46
291. Lee SB, Esteban M. 1994. *Virology* 199: 491–96
292. Lee SB, Rodriguez D, Rodriguez JR, Esteban M. 1997. *Virology* 231:81–88
293. Yeung MC, Liu J, Lau AS. 1996. *Proc. Natl. Acad. Sci. USA* 93:12451–55
294. Wada N, Matsumura M, Oba Y, Kobayashi N, Takizawa T, Nakanishi Y. 1995. *J. Biol. Chem.* 270:18007–12
295. Takizawa T, Ohashi K, Nakanishi Y. 1996. *J. Virol.* 70:8128–32
296. Stark GR, Dower WJ, Schimke RT, Brown RE, Kerr IM. 1979. *Nature* 278:471–73
297. Cohrs RJ, Goswami BB, Sharma OK. 1988. *Biochemistry* 27:3246–52
298. Reid TR, Hersh CL, Kerr IM, Stark GR. 1984. *Anal. Biochem.* 136:136–41
299. Ilson DH, Torrence PF, Vilcek J. 1986. *J. Interferon Res.* 6:5–12
300. Hovanessian AG, Laurent AG, Chebath J, Galabru J, Robert N, Svab J. 1987. *EMBO J.* 6:1273–80
301. Hovanessian AG, Svab J, Marie I, Robert N, Chamaret S, Laurent AG. 1988. *J. Biol. Chem.* 263:4945–49
302. Marie I, Blanco J, Rebouillat D, Hovanessian AG. 1997. *Eur. J. Biochem.* 248:558–66
303. Ball LA, White CN. 1979. *Regulation of Macromolecular Synthesis by Low Molecular Weight Mediators*, ed. H Koch, D Richter, pp. 303–17. New York: Academic
304. Justesen J, Ferbus D, Thang MN. 1980. *Proc. Natl. Acad. Sci. USA* 77:4618–22
305. Cayley PJ, Kerr IM. 1982. *Eur. J. Biochem.* 122:601–8
306. Justesen J, Worm-Leonhard H, Ferbus D, Petersen HU. 1985. *Biochimie* 67: 651–55
307. Turpaev K, Hartmann R, Kisselev L, Justesen J. 1997. *FEBS Lett.* 408:177–81
308. Cayley PJ, Davies JA, McCullagh KG, Kerr IM. 1984. *Eur. J. Biochem.* 143: 165–74
309. Matsuyama T, Grossman A, Mittrucker HW, Siderovski DP, Kiefer F, et al. 1995. *Nucleic Acids Res.* 23:2127–36
310. Eisenbeis CF, Singh H, Storb U. 1995. *Genes Dev.* 9:1377–87
311. Briken V, Ruffner H, Schultz U, Schwarz A, Reis LFL, et al. 1995. *Mol. Cell. Biol.* 15:975–82
312. Matsuyama T, Kimura T, Kitagawa M, Pfeffer K, Kawakami T, et al. 1993. *Cell* 75:83–97
313. Reis LF, Ruffner H, Stark G, Aguet M, Weissmann C. 1994. *EMBO J.* 13:4798–806
314. White LC, Wright KL, Felix NJ, Ruffner H, Reis LF, et al. 1996. *Immunity* 4:365–76
315. Lohoff M, Ferrick D, Mittrucker HW, Duncan GS, Bischof S, et al. 1997. *Immunity* 6:681–89
316. Taki S, Sato T, Ogasawara K, Fukuda T, Sato M, et al. 1997. *Immunity* 6:673–79
317. Kamijo R, Harada H, Matsuyama T, Bosland M, Gerecitano J, et al. 1994. *Science* 263:1612–15
318. Tamura T, Ishihara M, Lamphier MS, Tanaka N, Oishi I, et al. 1995. *Nature* 376:596–99
319. Holtschke T, Lohler J, Kanno Y, Fehr T, Giese N, et al. 1996. *Cell* 87:307–17