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21. Because of the finite curvature of the surfaces, a small beam size is required to probe ultrathin liquids of order a few molecular layers.
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Functional Role of Type I and Type II Interferons in Antiviral Defense

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Mice lacking the known subunit of the type I interferon (IFN) receptor were completely unresponsive to type I IFNs, suggesting that this receptor chain is essential for type I IFN–mediated signal transduction. These mice showed no overt anomalies but were unable to cope with viral infections, despite otherwise normal immune responses. Comparison of mice lacking either type I or type II IFN receptors showed that, at least in response to some viruses, both IFN systems are essential for antiviral defense and are functionally nonredundant.

Interferons were discovered on the basis of their antiviral activity. Two families of IFNs can be distinguished: type I IFNs (IFN-α, IFN-β, and IFN-ω), which are encoded by a family of over 20 genes (*1*), and type II IFN (IFN-γ), which is structurally unrelated and encoded by a single gene (*2*). In addition to their antiviral properties, type I and type II IFNs exert widely overlapping pleiotropic effects including inhibition of cell growth, antitumor action, involvement in hematopoiesis, and regulatory effects on cellular and humoral immune responses (*3*) which may be pathogenic in certain autoimmune diseases (*4*). The biological relevance of these nonantiviral effects remains elusive. The isolation of a trophoblast IFN (IFN-ω) from ruminants revived the question of whether IFNs are also engaged in embryonic development (*5*).

To elucidate the physiological role of the type I IFN system, we generated mice with a deficient type I IFN system. Because inactivation of the entire type I IFN gene family was unfeasible, the gene encoding the known receptor subunit was chosen as a target. Even though type I IFNs were shown to cross-compete for receptor binding (*6*, *7*), some evidence suggested that several type I receptors may exist (*8*, *9*). Still, reconstitution of an IFN-resistant murine cell line with the known receptor subunit indicated that this receptor component might be essential for the response to IFN-α/β (*10*). Inactivation of the type I IFN receptor gene in embryonic stem (ES) cells was achieved by homologous recombination (*11*) as described in Fig. 1. The IFN-α/β$R^{0/0}$ mice were obtained with a mendelian frequency, proved fertile, and had no apparent phenotypic anomalies by 6 months of age. Northern (RNA) blot analysis of total RNA from primary embryonic fibroblasts (PMEFs) hybridized with the complete murine IFN-α/βR complementary DNA (cDNA) as a probe revealed a 4.5-kb mRNA for wild-type cells (*10*), whereas no transcript was detected for mutant cells (*12*), possibly because of instability of the truncated, mutant mRNA.

As expected, the type I receptor was functionally inactive, because PMEFs from IFN-α/β$R^{0/0}$ embryos were unresponsive to the antiviral action of natural murine type I IFN, a mixture of IFN-α and IFN-β (Fig. 2A). However, mutant cells remained sensitive to IFN-γ, although we observed one-fourth to one-eighth the sensitivity as compared with wild-type cells. Functional inactivation of the type I receptor was further confirmed by Northern blot analysis of the RNA from PMEFs with probes derived from several different IFN-inducible genes (Fig. 2B). Mutant cells were unresponsive to natural type I IFNs, recombinant murine IFN-β, and recombinant human IFN-α_2/α_1 to which normal mouse cells are responsive (*13*). In this assay the response to murine IFN-γ was indistinguishable from that of wild-type cells. We found no evidence for a functional alteration of the IFN-γ system in IFN-α/β$R^{0/0}$ mice. Thus, IFN-α/β$R^{0/0}$ mice were as resistant to *Listeria monocytogenes* as wild-type mice, whereas IFN-γ$R^{0/0}$ mice were highly susceptible to this pathogen (*14*).

To monitor the response to type I IFN in vivo, we analyzed the induction of the *Mx-1* gene, a strictly type I IFN–specific response marker in mouse cells (*15*). Mutant and wild-type animals were injected intraperitoneally with either the IFN inducer poly(I)·poly(C) (PIC; Fig. 2C) or recombinant human IFN-α_2/α_1 (10^5 U), or they were infected intravenously with 2×10^6 plaque-forming units (PFU) of vesicular stomatitis virus (VSV). RNA from various tissues was isolated after 7.5 hours (IFN-α_2/α_1 and PIC)

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or 24 hours (VSV) and analyzed by Northern blotting. In contrast to wild-type mice, Mx-1 mRNA remained undetectable in any of the organs of mutant mice (Fig. 2C), regardless of which inducer was used. Even assuming that various forms of a type I IFN receptor might exist, these results show that the cloned receptor chain is an essential signaling component for the response to all type I IFNs tested.

Type I IFNs are produced by the early preimplantation embryo of certain ruminants (*5*) and were found in mouse placentas (*16*). The absence of an overt phenotype in mice lacking the type I IFN receptor argues against an essential role of type I IFNs in murine embryonic development or feto-maternal interaction, even though we have not formally shown that these particular IFN subtypes are inactive in IFN-α/βR$^{0/0}$ mice.

Type I IFNs have been discussed as natural regulators of cell growth and differentiation, notably in hematopoiesis (*17*). Certain bone marrow–derived progenitors seem particularly sensitive to IFN-α, and accordingly, certain leukemias, such as hairy cell leukemia and chronic myeloid leukemia, respond well to IFN-α therapy (*18–20*). The hematological status of 4- to 12-week-old IFN-α/βR$^{0/0}$ mice revealed a two- to threefold increased level of circulating mature monocytes and neutrophils as the only aberration. Because this anomaly may be due to subclinical infections, further investigations in animals kept under more stringent hygienic conditions will be required. Comparative cytofluorometry revealed no anomalies in the major lymphocyte subsets in terms of expression of CD3, CD4, CD8 (thymocytes and splenocytes), and major histocompatibility complex (MHC) class I and class II antigens (thymocytes, splenocytes, and peritoneal macrophages). Only cell surface immunoglobulin M (IgM) was slightly reduced in mutant animals.

To assess the consequences of an IFN-α/β receptor null mutation on host resistance to infection, we inoculated mutant and control mice with VSV (*21*), Semliki Forest virus (SFV), vaccinia virus (*22*), or lymphocytic choriomeningitis virus (LCMV) (*23*) and compared the antiviral response with that of mice lacking IFN-γ receptors (*14*). Mice devoid of type I IFN receptors were extremely susceptible to these viral infections. Whereas the median lethal dose (LD_{50}) for intravenously injected VSV is normally in the range of 10^8 PFU, mutant mice died within 3 to 6 days after infection with only 30 to 50 PFU (Table 1). VSV replication occurred in all organs tested, with titers that increased by 10^2- to 10^6-fold reaching up to 10^8 PFU per

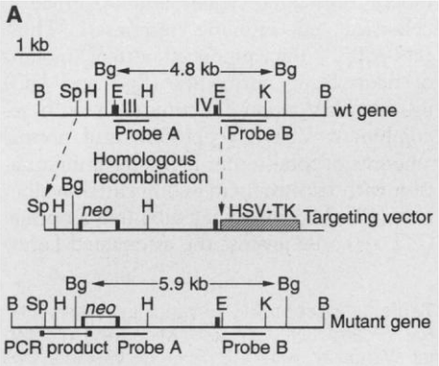

Fig. 1. Gene targeting of the murine IFN-α/βR gene and germline transmission of the disrupted allele. (**A**) A replacement vector containing exons III and IV and encompassing 3.6 kb of genomic sequence isolated from an isogenic library (derived from 129Sv/Ev-ES cells) was constructed by inserting a blunted Xho I–Bam HI fragment of pMC1neopA (Stratagene) into the blunted Eco RI site of exon III, corresponding to amino acid 88 of the translated reading frame. To allow negative selection against random integration, we introduced a HSV-TK gene cassette (*11*). Exons are indicated by black boxes. B, Bam HI; Bg, Bgl II; E, Eco RI; H, Hind III; K, Kpn I; Sp, Sph I; wt, wild-type. D3 ES cell clones (*35*) that had undergone homologous recombination were screened by polymerase chain reaction (PCR) as described (*30*) with the depicted PCR-primer pair. The frequency of homologous recombination was 1 in 26 G-418 and FIAU-resistant colonies. (**B**) Southern (DNA) blot analysis of Bgl II–digested genomic tail DNA. The probe was an 0.8-kb Eco RI–Hind III fragment (probe A). Lane 1, wild-type; lane 2, IFN-α/βR$^{+/0}$ heterozygote; lane 3, IFN-α/βR$^{0/0}$ homozygote. No ectopic or tandem integration events were detected with either probe A or B. Sizes are indicated in kilobases.

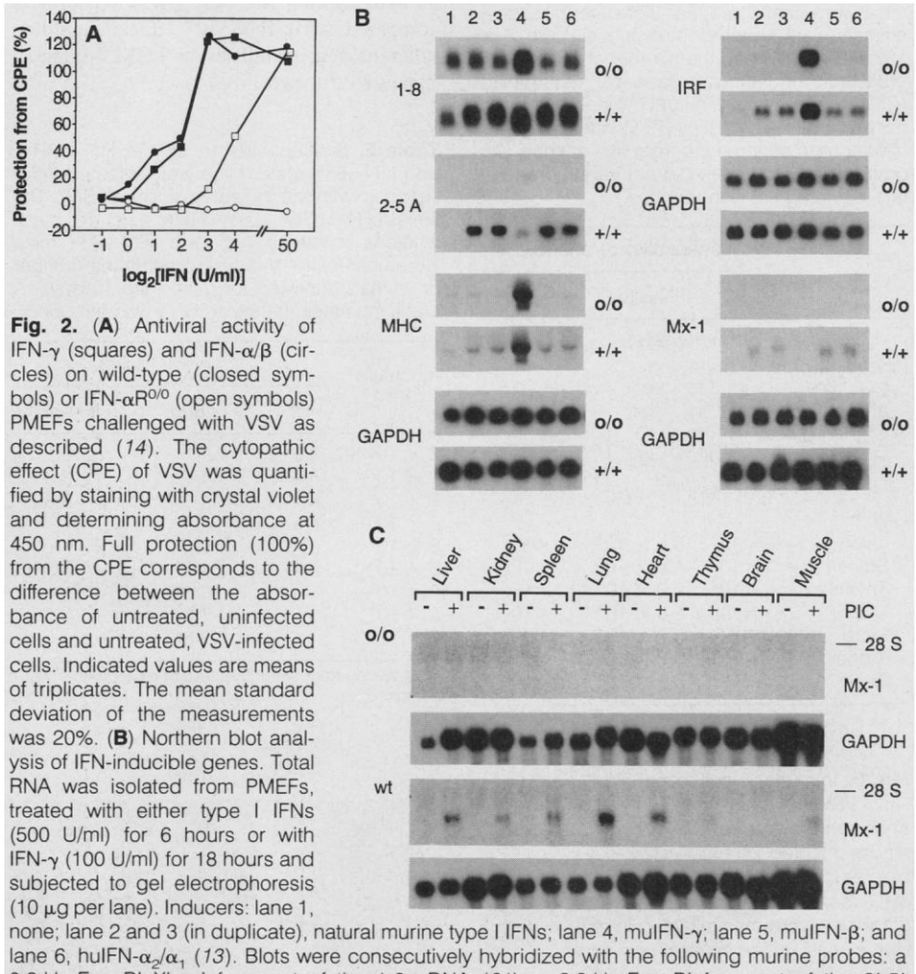

Fig. 2. (**A**) Antiviral activity of IFN-γ (squares) and IFN-α/β (circles) on wild-type (closed symbols) or IFN-αR$^{0/0}$ (open symbols) PMEFs challenged with VSV as described (*14*). The cytopathic effect (CPE) of VSV was quantified by staining with crystal violet and determining absorbance at 450 nm. Full protection (100%) from the CPE corresponds to the difference between the absorbance of untreated, uninfected cells and untreated, VSV-infected cells. Indicated values are means of triplicates. The mean standard deviation of the measurements was 20%. (**B**) Northern blot analysis of IFN-inducible genes. Total RNA was isolated from PMEFs, treated with either type I IFNs (500 U/ml) for 6 hours or with IFN-γ (100 U/ml) for 18 hours and subjected to gel electrophoresis (10 μg per lane). Inducers: lane 1, none; lane 2 and 3 (in duplicate), natural murine type I IFNs; lane 4, muIFN-γ; lane 5, muIFN-β; and lane 6, huIFN-α_2/α_1 (*13*). Blots were consecutively hybridized with the following murine probes: a 0.2-kb Eco RI–Xba I fragment of the 1-8 cDNA (*31*); a 2.2-kb Eco RI fragment of the 2′-5′ A-synthetase gene (*32*); a 1.8-kb genomic Xba I fragment of the H-2D^b MHC class I gene (*33*); a 1.1-kb Nco I fragment of the IRF-1 cDNA (*34*), an antisense RNA probe of the murine Mx-1 cDNA (*15*), and a 0.5-kb Xho II fragment of the glyceraldehyde-3-phosphate-dehydrogenase gene (*35*). Experiments were replicated with RNA from independent embryos. (**C**) Mice were injected intraperitoneally with 200 μg of poly(I).poly(C) (PIC), and after 7.5 hours, total RNA (10 μg per lane) from various organs was analyzed by Northern blotting with an antisense RNA probe of murine Mx-1 cDNA; 28 S, 28*S* RNA.

gram of tissue (Table 1 and Fig. 3). A similar susceptibility was seen after infection with SFV; mutant mice were also approximately 10^6-fold more sensitive to the virus than wild-type mice (Table 2). The in vivo relevance of the antiviral action of type I IFN had been documented by the use of antibodies to IFN (*24–26*). The inactivation of the type I IFN receptor proved the essential role the type I IFN system plays in antiviral defense.

In mice inoculated with either VSV or SFV, the type II IFN system did not appear to influence the course of the infection, because mice lacking the IFN-γ receptor (*14*) showed no increased susceptibility to these viruses as compared with control mice (Tables 1 and 2). In contrast, mice lacking either the type I or the type II receptor succumbed to infection with 10^6 PFU of vaccinia virus within 5 to 6 days, and in both mutant strains this virus replicated to titers 10^3- to 10^5-fold as high as those in wild-type mice (Table 3). These results indicate that, in vivo, IFN-α/β and IFN-γ may exert their antiviral activities through different, partially nonredundant pathways.

A similar complementarity of the two IFN systems was observed when mutant mice were challenged with the noncytopathic LCMV-WE strain (*23*). Inoculation of 3×10^2 PFU into the footpad of either IFN-α/βR$^{0/0}$ or IFN-γR$^{0/0}$ mice resulted in a generalized virus spread with viral titers increased 10^4- to 10^5-fold for IFN-α/βR$^{0/0}$ animals and 10- to 10^4-fold for IFN-γR$^{0/0}$ mice (Table 4). Virus replication was more pronounced in the thymus and brain of animals lacking the type I IFN receptor as compared with IFN-γR$^{0/0}$ mice, signaling different susceptibilities of LCMV-infected tissues to the two IFNs.

In contrast with control mice, IFN-α/βR$^{0/0}$ animals did not mount a detectable cytotoxic T lymphocyte (CTL) response against LCMV after infection with 3×10^2 pfu into the footpad, nor did they show any CD8$^+$ T cell–dependent footpad swelling after local infection. Correspondingly, though intracerebral inoculation with 100 PFU of the neuropathic LCMV-Armstrong strain caused immunopathology and was lethal for control mice (*27*), none of the four IFN-α/βR$^{0/0}$ mice was affected. The course of LCMV infection in IFN-α/βR$^{0/0}$ mice and the lack of a measurable CTL response was reminiscent of the exhaustion of virus-specific activated T cells in wild-type animals infected with high doses of rapidly replicating lymphotropic LCMV (*27*). The lack of immune response in LCMV-infected IFN-α/βR$^{0/0}$ mice was not due to a deficiency in the immune system. Like IFN-γR$^{0/0}$ mice, mice lacking the type I IFN receptor were able to mount normal T helper and cytotoxic T cell–mediated immune responses. Thus, IFN-α/βR$^{0/0}$ mice produced normal amounts of neutralizing antibodies (IgM and IgG) against VSV upon immunization with recombinant VSV glycoprotein and normal amounts of total serum IgG after immunization with trinitrophenyl-conjugated ovalbumin (*12*). Likewise, they mounted a normal CTL response against the attenuated Lancy

Table 1. Susceptibility to VSV in IFN-α/βR$^{0/0}$ and IFN-γR$^{0/0}$ mice. Mice were infected intravenously with the indicated titers of VSV (Indiana strain). The IFN-α/βR$^{0/0}$ mice died within 3 to 6 days, whereas wild-type (wt) and IFN-γR$^{0/0}$ mice were unaffected. For titer determinations, animals were infected intravenously with 2×10^6 PFU of VSV, and the number of PFU recovered from the various organs was determined (*21*) at day 4 (for IFN-α/βR$^{0/0}$ and wild-type mice) or day 6 (for IFN-γR$^{0/0}$) after infection. Values represent means from two animals per group. Individual titers did not vary more than one log; n.d., not done.

	Susceptibility of phenotype		
	wt	IFN-α/βR$^{0/0}$	IFN-γR$^{0/0}$
	Survival (days)		
Inoculum (pfu)			
2×10^6	3/3	0/3	3/3
3×10^1 to 5×10^1	6/6	0/6	n.d.
$<3 \times 10^1$	6/6	6/6	n.d.
	Viral titers (PFU/g)		
Organ			
Ovary	$<10^2$	8.5×10^8	$<10^2$
Spleen	$<10^2$	1.8×10^8	$<10^2$
Thymus	$<10^2$	2.0×10^8	$<10^2$
Liver	$<10^2$	2.0×10^8	$<10^2$
Lung	$<10^2$	5.0×10^7	$<10^2$
Kidney	$<10^2$	3.0×10^7	$<10^2$
Brain	$<10^2$	5.0×10^4	$<10^2$

Table 2. Susceptibility to SFV in IFN-α/βR$^{0/0}$ and IFN-γR$^{0/0}$ mice. Mice were infected intravenously with the indicated doses of SFV. The IFN-α/βR$^{0/0}$ mice died within 4 to 10 days, whereas wild-type (wt) and IFN-γR$^{0/0}$ mice were unaffected. Values represent the number of animals surviving for more than 10 days. A blank indicates the experiment was not done.

Inoculum (PFU)	Survival (days)		
	wt	IFN-α/βR$^{0/0}$	IFN-γR$^{0/0}$
2×10^8	8/8*		
2×10^7	4/4		3/3
2×10^6	4/4		3/3
2×10^5	4/4		
2×10^4		0/4	
2×10^3	4/4	1/8*	3/3
2×10^2		1/8*	
2×10^1		7/8*	
2×10^0		7/8*	

*The values from two independent experiments were compiled.

Table 3. Susceptibility to vaccinia virus in IFN-α/βR$^{0/0}$ and IFN-γR$^{0/0}$ mice. Mice were infected intravenously with 10^6 PFU of vaccinia WR virus, and the number of PFU recovered from the various organs was determined (*22*) at day 4 after infection. Values represent means from four animals per group. Individual titers did not vary more than one log.

Organ	Viral titers (PFU/g)		
	wt	IFN-α/βR$^{0/0}$	IFN-γR$^{0/0}$
Ovary	1.1×10^5	4.6×10^8	3.2×10^8
Spleen	1.0×10^3	4.1×10^6	1.7×10^6
Liver	1.0×10^1	1.7×10^7	3.6×10^5
Lung	9.5×10^4	4.8×10^7	5.7×10^7

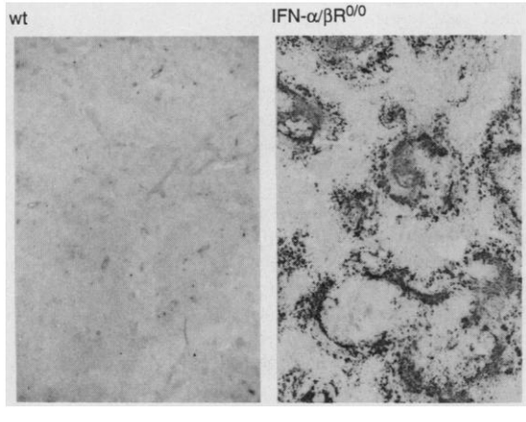

Fig. 3. Immunohistochemical analysis of VSV replication in the spleen of a wild-type (wt) and an IFN-α/βR$^{0/0}$ mouse infected intravenously 2 days before with 10^6 PFU of VSV (*36*). Cells of the marginal zones and B cell areas of follicles were most affected in mutant animals. In the controls only very few single cells, not visible at this magnification, were stained in the marginal zones. Magnification, ×36.

Table 4. Susceptibility to LCMV in IFN-α/βR$^{0/0}$ and IFN-γR$^{0/0}$ mice. Mice were infected with 3×10^2 PFU of LCMV (WE strain) into the footpad, and the number of pfu recovered from the various organs was determined (*23*) at day 10 after infection. Values represent means from two animals per group. Individual titers did not vary more than one log.

Organ	Viral titers (PFU/g)		
	wt	IFN-α/βR$^{0/0}$	IFN-γR$^{0/0}$
Ovary	$<10^2$	1.18×10^6	1.00×10^6
Spleen	$<10^2$	1.24×10^7	4.00×10^3
Liver	$<10^2$	1.50×10^6	1.50×10^5
Lung	$<10^2$	1.00×10^6	1.00×10^3

strain of vaccinia virus (*12*).

These findings suggest that a deficiency in innate rather than acquired immunity accounts for the susceptibility of IFN-α/βR$^{0/0}$ mice to viral infections. Even though natural killer (NK) cell activity in spleens of animals pretreated with IFN-γ was indistinguishable in wild-type and IFN-α/βR$^{0/0}$ mice, we observed that upon infection by LCMV the NK cell response was reduced by about 50% in IFN-α/βR$^{0/0}$ mice. Several aspects of IFN function still need to be addressed, and it will be interesting to see whether these mice show an impaired antitumor defense. So far we have not observed an increased rate of spontaneous tumor incidence up to 6 months of age.

Mice that lack the type I IFN receptor revealed the primordial role of the type I IFN system as a tightly regulated response system functioning predominantly in early antiviral defense. Comparison of mice lacking either the type I or type II IFN system starts to reveal the functional complementarity of both IFN systems. Whereas the antiviral defense against some viruses such as VSV or SFV seems to involve primarily the type I IFN system, the defense against other viruses such as vaccinia virus or LCMV requires a cooperation of both systems. It is well established and illustrated in Fig. 2B that type I and type II IFNs induce in part the same genes, although to a different extent (*28*). Thus, MHC class I or interferon regulatory factor–1 (IRF-1) transcripts were strongly induced by type II IFN and much less by type I IFN, 1-8 mRNA was induced to similar levels, whereas Mx-1 mRNA was preferentially induced by type I IFN. As in the case of the Mx-1 protein, which is remarkably virus specific (*29*), such differences may qualitatively affect antiviral responses elicited by type I compared with type II IFN.

Mice with a combined IFN-α/β- and IFN-γ receptor deletion obtained by breeding are expected to become even more susceptible to certain viruses. These animals may allow researchers to isolate and investigate pathogens that are otherwise hidden or difficult to identify, including viruses possibly involved in chronic, immunopathological, and autoimmune diseases.

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Involvement of the IRF-1 Transcription Factor in Antiviral Responses to Interferons

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The mechanisms underlying interferon (IFN)-induced antiviral states are not well understood. Interferon regulatory factor–1 (IRF-1) is an IFN-inducible transcriptional activator, whereas IRF-2 suppresses IRF-1 action. The inhibition of encephalomyocarditis virus (EMCV) replication by IFN-α and especially by IFN-γ was impaired in cells from mice with a null mutation in the *IRF-1* gene (IRF-1$^{-/-}$ mice). The IRF-1$^{-/-}$ mice were less resistant than normal mice to EMCV infection, as revealed by accelerated mortality and a larger virus titer in target organs. The absence of IRF-1 did not clearly affect replication of two other types of viruses. Thus, IRF-1 is necessary for the antiviral action of IFNs against some viruses, but IFNs activate multiple activation pathways through diverse target genes to induce the antiviral state.

The type I IFNs (IFN-α and IFN-β) are expressed in many cells upon viral infection, whereas type II IFN (IFN-γ) is produced by activated T lymphocytes and natural killer cells. The IFNs confer cellular resistance to many types of viruses by activating a set of IFN-inducible genes, but the mechanism underlying the IFN-mediated establishment of the antiviral state is still not fully understood (*1*). Two structurally related transcription factors, IRF-1 and IRF-2, have been identified that bind to the same DNA sequence elements (IRF-Es) in the *IFN-α* and *IFN-β* promoters (*2–4*). These two factors also bind to the IFN-stimulated regulatory elements (ISREs) found in many IFN-inducible gene promot-

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