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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ers (*3, 5*). The ISREs are also bound by other transcription factors such as IFN-stimulated gene factor–3 (ISGF-3) and IFN consensus sequence binding protein (ICSBP) (*6*). IRF-1 functions as a transcriptional activator, and IRF-2 suppresses IRF-1 function (*3, 7*). Furthermore, the *IRF-1* gene per se is inducible by IFNs (*3, 4, 8*), which suggests that IRF-1 is involved in IFN-mediated cellular responses (*7, 9*). Mice with a null mutation in the *IRF-1* gene (IRF-$1^{-/-}$) or *IRF-2* gene (IRF-$2^{-/-}$) have been generated by gene targeting in embryonic stem cells (*10*). The induction of the inducible nitric oxide synthase gene (*iNOS*) by IFNs was inhibited in the IRF-$1^{-/-}$ macrophages (*11*), whereas the type I IFN–induced activation of the genes for 2′-5′ oligoadenylate synthetase and double-stranded RNA-dependent protein kinase genes (*2-5A synthetase* and *PKR*, respectively) was not affected in embryonic fibroblasts (EFs) from either the IRF-$1^{-/-}$ or IRF-$2^{-/-}$ mice (*10*). In view of the reported participation of the *iNOS, 2-5A synthetase*, and *PKR* genes in IFN-mediated inhibition of viral replication (*12, 13*), it has remained unclear if and how IRF-1 functions in the antiviral actions of IFNs.

Embryonic fibroblasts from wild-type IRF-$1^{-/-}$ mice were first treated with either IFN-α or IFN-γ and challenged with encephalomyocarditis virus (EMCV) (Picornaviridae), and the viral yield was determined (Fig. 1A) (*14*). Viral replication was inhibited in a concentration-dependent fashion in EFs from wild-type mice pretreated with IFN-α or with IFN-γ. In contrast, this inhibition was impaired in EFs from IRF-$1^{-/-}$ mice. In cells treated with IFN-α at a concentration of 250 or 500 U/ml, the difference in virus yield between the wild-type and IRF-$1^{-/-}$ EFs was about 10-fold or more. However, this difference became marginal when the IFN concentration was increased to 1000 U/ml. The antiviral effect of IFN-γ in IRF-$1^{-/-}$ EFs was impaired more severely; the virus yield in IRF-$1^{-/-}$ EFs was 100- to 500-fold greater than that in wild-type EFs, even at the highest IFN concentration used. These results were reproducible with EFs derived from other littermates, and similar differences in the antiviral actions of IFN-α and IFN-γ were also seen in peritoneal macrophages from wild-type and IRF-$1^{-/-}$ mice by virus yield reduction assay with EMCV (*15*). Consistent with these observations, protection from the cytopathic effects of EMCV by IFN-α (250 U/ml) or by IFN-γ (25 U/ml) was 16-fold or 1000-fold more effective, respectively, in wild-type EFs than in IRF-$1^{-/-}$ EFs (*15*).

IRF-1 and IRF-2 act in a mutually antagonistic manner. IRF-2 suppresses IRF-1 action by competing for binding to the IRF-Es or ISREs (*3, 5, 7*). Because other IFN-inducible factors such as ISGF-3 and ICSBP also bind to the ISREs (*6*), it is conceivable that the function of these factors is also affected by IRF-2, possibly in a more profound manner in the absence of IRF-1. Hence, we addressed the question of whether the impaired antiviral action is due to the lack of IRF-1–mediated events or a result of the dominant repressor function of IRF-2 in the absence of IRF-1. EFs from the IRF-$2^{-/-}$ mice were resistant to EMCV upon IFN treatment, whereas the defect in the IFN response seen in IRF-$1^{-/-}$ EFs was also observed with EFs from the mice lacking both *IRF-1* and *IRF-2* genes (Fig. 1B) (*16*). Thus, IRF-1 itself mediates the establishment of the IFN-induced antiviral state against EMCV.

We compared the course of EMCV infection in wild-type and IRF-$1^{-/-}$ mice. After intraperitoneal infection with EMCV, the survival time of the mutant mice was shorter than that of the wild-type mice (Fig. 2A). Likewise, infection with another picornavirus, coxsackievirus B3, was lethal to IRF-$1^{-/-}$ mice but not to wild-type mice (*17*). We also examined EMCV titers 3 days after the infection in the target organs of this virus, the heart and brain (*18*), and statistically significant differences in the amounts of the virus were detected between the wild-type and IRF-$1^{-/-}$ mice in both organs (Fig. 2B) (*15*). Although these phenotypic differences may represent the consequence of complex host defense mechanisms (*18*), our observations are consistent with a deficiency in IFN action against EMCV in the IRF-$1^{-/-}$ cells in vitro.

We also analyzed IFN-induced cellular resistance to vesicular stomatitis virus (VSV; Rhabdoviridae) and herpes simplex virus (HSV; Herpesviridae). After infection with VSV, no difference in virus yield was seen between wild-type and IRF-$1^{-/-}$ EFs, whereas a small but reproducible difference was observed after HSV infection, particularly after IFN-γ treatment (Fig. 3).

The mechanism whereby IRF-1 leads to the establishment of the antiviral state is not clear. Several IFN-inducible genes have been proposed as mediators of the antiviral action of IFNs; for example, overexpression of the *PKR* or *2-5A synthetase* complementary DNAs (cDNAs) confers cellular resistance against EMCV but not against VSV and HSV (*12*), and overexpression of a dominant negative mutant of the *2-5A*–

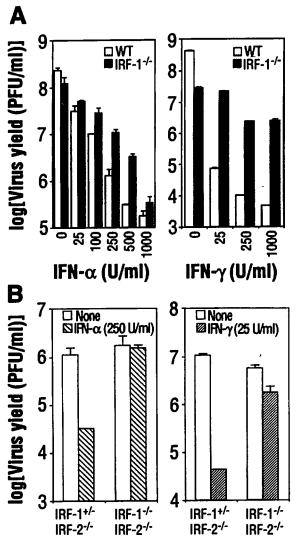

Fig. 1. (**A**) Inhibition of EMCV replication by IFN-α or IFN-γ treatment in EFs from IRF-$1^{-/-}$ mice. The EFs from wild-type (WT) mice or IRF-$1^{-/-}$ mice of the same litters were treated with either IFN-α or IFN-γ for 18 hours and subsequently infected with EMCV. After 24 hours, the virus yield was determined by a plaque assay (*14*). The mean ± SD of virus titers (expressed in logarithms) in duplicate cultures was calculated. The titers of adsorbed viruses were about the same for both wild-type and IRF-$1^{-/-}$ EFs [approximately 10^4 plaque-forming units (PFU) per milliliter. Thus, the difference of the virus yield represents the extent of viral replication. (**B**) Inhibition of EMCV replication by IFN-α or IFN-γ in EFs from IRF-$2^{-/-}$ mice and mice lacking both *IRF-1* and *IRF-2* genes. The EFs of the same litters were treated with IFN-α (250 U/ml) or IFN-γ (25 U/ml) before EMCV infection, and the virus yield was determined as above. These results were essentially reproducible with EFs derived from other littermates.

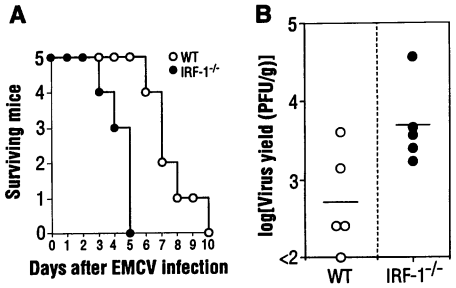

Fig. 2. (**A**) Survival of wild-type and IRF-$1^{-/-}$ mice after EMCV infection. Five wild-type and IRF-$1^{-/-}$ mice each were infected intraperitoneally with EMCV (10^5 PFU), and survival of these mice was monitored daily. (**B**) Titers of viruses recovered from the hearts of EMCV-infected mice. Five wild-type and IRF-$1^{-/-}$ mice each were infected intraperitoneally with EMCV (10^6 PFU). Three days later, the titers of the virus in the hearts were determined by a plaque assay (*14, 18*). The values for individual mice are shown (in logarithms). In this experiment, two IRF-$1^{-/-}$ mice were moribund on day 3. Horizontal bars indicate mean values. The difference in the virus titers between those in the wild-type and those in IRF-$1^{-/-}$ mice was statistically significant ($P < 0.02$ by Student's t test).

dependent RNase cDNA suppresses type I IFN–induced inhibition of EMCV replication (*19*). Overexpression of *iNOS* cDNA also induces an antiviral state to various viruses, including HSV (*13*). In addition, guanylate-binding protein (*GBP*), *9-27*, and *1-8* have been implicated in the antiviral response (*20*). In the case of VSV, a unique mechanism has been proposed whereby the IFN-induced soluble form of the low density lipoprotein receptor inhibits viral replication (*21*). As shown in Fig. 4, analysis of mRNA expression in wild-type and IRF-$1^{-/-}$ EFs revealed several things. (i) Induction of the *PKR*, *9-27* and *1-8* genes by either IFN-α or IFN-γ was similar in wild-type and IRF-$1^{-/-}$ EFs. (ii) Induction of the *2-5A synthetase* gene by IFN-γ was weak compared to its induction by IFN-α in wild-type EFs, and the former induction was diminished in IRF-$1^{-/-}$ EFs. (iii) Induction of the *GBP* gene by IFN-α was mildly impaired (2.5-fold at peak induction), and induction by IFN-γ was severely impaired (40-fold at peak induction) in IRF-$1^{-/-}$ EFs (*22*). (iv) The *iNOS* gene was weakly induced by IFN-γ but not by IFN-α in wild-type EFs, and this induction was completely abrogated in IRF-$1^{-/-}$ EFs. Thus, dependency on IRF-1 varies among these genes, with *GBP* and *iNOS* gene induction being strongly IRF-1–dependent. It is possible that in the case of the *GBP* gene induction by IFN-α, both IRF-1 and ISGF-3 may participate during the course of induction (*23*). The role of these genes as effectors of the antiviral action of IRF-1 remains to be clarified.

Our data also suggest that IRF-1 may be more important in mediating the antiviral effects of IFN-γ than those of IFN-α, as induction of the *iNOS*, *GBP*, and *2-5A synthetase* genes by IFN-γ is impaired in IRF-$1^{-/-}$ EFs. We also note that the *IRF-1* gene is efficiently induced over a more prolonged period by IFN-γ than by IFN-α both in cultured cell lines (*8*) and in the wild-type EFs (*15*). However, loss of IRF-1 does have some effects on the antiviral state induced by IFN-α (Fig. 1A). Because the genes induced by IFN-α were relatively unaffected by loss of IRF-1, we infer that there exist other, as yet unidentified, target genes mediating the antiviral effects of IRF-1.

Our results also indicate that the IRF-1–mediated antiviral action of IFNs is selective for some viruses. Other transcription factors, such as type I IFN–inducible ISGF-3 and IFN-γ–inducible γ activated factor (GAF), presumably mediate IFN actions that are unaffected in IRF-$1^{-/-}$ cells (*6*, *24*). Overexpression of the *IRF-1* cDNA, however, induces an antiviral state to various viruses, including VSV, EMCV, and Newcastle disease virus (*9*, *25*). Presumably, the difference between these results and our present studies stems from differences between experimental systems—that is, overexpression versus disruption of the *IRF-1* gene. Studies of overexpression can clarify the potential of certain gene products but do not indicate whether they are essential under physiological conditions. Our study reveals that IFNs activate IRF-1–dependent and –independent pathways to induce different antiviral states against different types of viruses, which further points to the importance of the combined action of diverse gene products for cellular resistance to viruses (*1*). IRF-$1^{-/-}$ mice are sensitive to mycobacterial infection (*11*), and IRF-1 can also act as a tumor suppressor (*26*). Thus, IRF-1 contributes to antiviral, antibacterial, and antitumor functions.

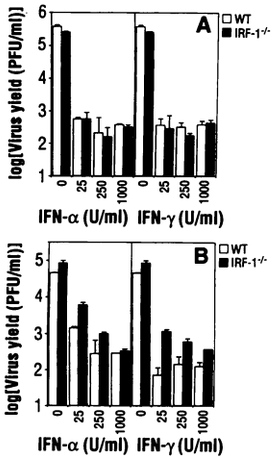

Fig. 3. Inhibition of VSV (**A**) and HSV (**B**) replication by IFN-α or IFN-γ in IRF-$1^{-/-}$ EFs. The EFs from wild-type or IRF-$1^{-/-}$ mice of the same litters were treated with IFN-α or IFN-γ for 18 hours and subsequently infected with VSV or HSV. The virus yield was determined as described (Fig. 1). The titers of adsorbed viruses were about the same for the wild-type and IRF-$1^{-/-}$ EFs (approximately $10^{1.5}$ PFU per milliliter in both viruses), indicating that the difference of virus yield represents the extent of viral replication. These results were essentially reproducible with EFs derived from other littermates.

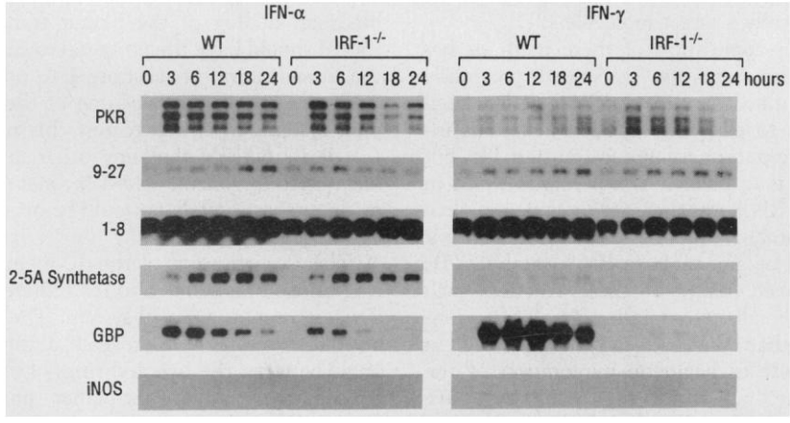

Fig. 4. Induction of several IFN-inducible gene mRNAs (*PKR, 9-27, 1-8, 2-5A synthetase, GBP*, and *iNOS*) by IFN-α or IFN-γ. The wild-type and IRF-$1^{-/-}$ EFs were treated with IFN-α (250 U/ml) or IFN-γ (25 U/ml). Total RNA was extracted at the indicated times, and 3 μg of RNA was subjected to RNA blotting analysis (*27*). Every filter was exposed to x-ray film for 20 hours [except the filters for *iNOS* (60 hours)] and reprobed with the *β-actin* probe to confirm the RNA content in each slot. These results were essentially reproducible with EFs derived from other littermates.

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14. The EFs were prepared from 12- to 14-day-old embryos, cultured in Dulbecco's modified minimum essential medium with 10% fetal calf serum, and seeded at 2.5×10^5 cells per well into 24-well plates. The cells were treated with immunoaffinity-purified natural murine IFN-α (Sumitomo Pharmaceutical, Osaka, Japan) or recombinant murine IFN-γ (Genzyme, Cambridge, MA) 18 hours before and throughout infection. EMCV, VSV, and HSV were allowed to adsorb for 1.5 hours at a concentration of 10, 0.01, and 0.1 plaque-forming units per cell, respectively, and the cells were washed twice with serum-free medium and replenished with 1 ml of medium. Half of the sample was harvested immediately to determine the titer of adsorbed viruses, and the remaining half was harvested 24 hours later to determine the titer of replicated viruses. After harvest, the cells were lysed by three cycles of freeze-thawing and sonication to recover EMCV and HSV or lysed by two cycles of freeze-thawing to recover VSV. Serially diluted virus samples were added to L cell or Vero cell monolayers to determine the yield of infectious virus in terms of plaque-forming units. After 2 to 3 days, viral plaques were counted after staining with 0.02% (w/v) neutral red. The IFNs present in the medium did not affect the efficiency of plaque formation on the L cell monolayer when virus samples were diluted more than 100 times.
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22. The *GBP* genes have been known as *GBP-1 (mag-1)* and *minor GBP* (including *mag-2*), and our probe can detect mRNA from both *GBP-1* and 2.5-kb *minor GBP* (*20*). Because these EFs do not express the *GBP-1* gene (*15*), the mRNA signal indicates expression of the 2.5-kb *minor GBP* gene.
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27. RNA blotting analysis was carried out as described (*7*). Probes for *PKR, 1-8, 2-5A synthetase*, and *β-actin* were prepared as described (*10*). The probe for *iNOS* was cloned from murine macrophage RNA. Probes for *GBP* and *9-27* were a 700-bp Bam HI–Eco RI fragment of pSP65-mGBP-1 and a 300-bp Spe I–Sty I fragment of Ldk927, respectively (*20*). The specific activity of each probe was approximately 5×10^8 cpm/μg.
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Expanding the Scope of RNA Catalysis

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The basic notions of transition state theory have been exploited in the past to generate highly selective catalysts from the vast library of antibody molecules in the immune system. These same ideas were used to isolate an RNA molecule, from a large library of RNAs, that catalyzes the isomerization of a bridged biphenyl. The RNA-catalyzed reaction displays Michaelis-Menten kinetics with a catalytic rate constant (k_{cat}) of 2.8×10^{-5} per minute and a Michaelis constant (K_m) of 542 μM; the reaction is competitively inhibited by the planar transition state analog with an inhibition constant (K_i) value of ~7 μM. This approach may provide a general strategy for expanding the scope of RNA catalysis beyond those reactions in which the substrates are nucleic acids or nucleic acid derivatives.

Many of the complex problems associated with biomolecular recognition and catalysis have been solved in nature by the generation and screening of large populations of molecules. This can occur on an evolutionary time scale or over the course of a few weeks during the immune response. One of the first examples in which the chemical potential of these processes was exploited was the use of transition state theory to select from the tremendous diversity of the immune system antibodies with catalytic activities (*1*). More recently, a number of methods have been developed for generating and screening large libraries of biological or synthetic molecules in vitro for their abilities to bind selectively or transform chemically a target molecule (*2*).

One application of these methods has been the search for RNAs with previously unidentified activities (*3*). RNA has been shown to efficiently catalyze reactions involving phosphoryl group transfers (*4*), but if one is to believe in a prebiotic world in which RNA was the primitive macromolecular catalyst, other basic chemical reactions should be amenable to RNA catalysis (*5*). Moreover, many of these reactions will require substrate binding by interactions other than Watson-Crick base pairing. In an effort to begin an exploration of the catalytic repertoire of RNA, we have screened a large library of RNA molecules for the ability to bind the near-planar transition state analog **3** and catalyze the isomerization of biphenyl **1** to its diastereomer **2** (Fig. 1).

The isomerization of substituted biphenyls is a well-characterized reaction involving rotation around a C–C sigma bond (Fig. 1) (*6*). Nonbonded interactions and angle strain in the planar transition state lead to a significant barrier to isomerization. As a result, isomeric substituted biphenyls can be isolated and interconvert quite slowly at room temperature. On the basis of Pauling's notion of enzymatic catalysis, in which maximum binding occurs to the transition state (TS‡) rather than to either substrates or products (*7*), an RNA that preferentially binds an analog of the planar transition state **4** should have the potential to catalyze the isomerization of substrate **1** to product **2**. This reaction provides one of the simplest systems in which to test this notion (*8*); it is unlikely that any other mechanisms such as general acid-base, metal ion, or electrostatic catalysis would be operative in this reaction.

The isomerization of the 10-membered ring bridged biphenyl **1** to its diastereomer **2** was chosen as a model system. The x-ray crystal structure of **2** reveals a dihedral angle between the two aryl rings of 68° (0° is coplanar). The near-planar phenanthrene derivative **3** was chosen as a mimic of the planar transition state **4** because bridged biphenyls of this sort are known to have dihedral angles of ~15° (*9*). In order to simplify substrate isolation and analysis of substrate to product ratios, we introduced

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