Characterization of the Interferon Regulatory Factor-7 and Its Potential Role in the Transcription Activation of Interferon A Genes

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The family of interferon regulatory factors (IRFs) plays an important role in modulating cellular responses to viral infection and cytokines, including IFNs. The transcription factors that are involved in the transcriptional activation of the IFNB gene have been extensively studied. However, the molecular mechanism by which virus activates the expression of the IFNA gene remains to be defined. Recently, we have identified a new IRF-7 isoform, denoted as IRF-7H, which encodes a protein of 514 amino acids and is most closely related to the IRF-3. The expression of IRF-7 is restricted to the lymphoid cell types and is inducible by virus, lipopolysaccharide, and IFNA. The functional characterization of IRF-7H reveals a presence of transactivation domain located carboxyl-terminal to its DNA binding domain. Overexpression of IRF-7H results in an activation of IFNA promoter in transient transfection assay and a strong enhancement of virus-mediated activation of this promoter. Whereas in uninfected cells, overexpressed IRF-7H is present mainly in the cytoplasm, viral infection facilitates the transfer of IRF-7H to the nucleus; overexpression of IRF-3 interferes with the virus-induced translocation of IRF-7H. Thus, IRF-7H exhibits functional similarity to IRF-3; however, the preferential expression of IRF-7 in lymphoid cells (the cell type that expresses IFNA) suggests that IRF-7 may play a critical role in regulating the IFNA gene expression.

The expression of IFNA genes in infected cells is regulated at the transcriptional level (1). Although the virus-induced signaling that leads to the activation of IFNA genes has not been well characterized, it was shown that the transcriptional activation of IFNA genes does not require de novo protein synthesis, and it is sensitive to several kinase inhibitors (2, 3). These observations suggested that a posttranscriptional modification, such as phosphorylation, of latent transcription factors, constitutes a vital part of virus-induced signaling pathway.

The cis-acting elements involved in the regulation of IFNA are located within 110 base pairs upstream from the transcription initiation site (4). In contrast to IFNB gene promoter, the cis regulatory region of IFNA promoters (inducible element (IE)) does not contain the NF-κB binding site (4); however, the virus response element of IFNA promoter was shown to bind multiple transcription factors (2). The study of transcriptional activation of murine IFNA4 promoter revealed that the binding of both AF-1-specific protein (p68 and p96) and of a member of the IRF family is required, because a point mutation in either IRF-E or AF-1 binding site abolished the virus-mediated induction of IFNA4 (5). Furthermore, a virus-induced complex/factor binding to a sequence outside IFNA4 IE as well as to positive regulatory domain I was detected (5); however, the proteins present in this complex have not been identified yet. Although the experimental evidence indicates that IRF-E in IFNA4 promoter is essential for its transcriptional activity in infected cells, it has been less clear which one of the IRF family members plays a critical role in the transcriptional activation of IFNA promoters.

The IRF family consists of a growing number of transcriptional factors that share homology in the amino-terminal 115 amino acids. This homologous region comprises the DNA binding domain (DBD) that is structurally related to Myb (6). Altogether, nine cellular IRFs have been identified. IRF-1, IRF-2, IRF-3, and p48 (interferon-stimulated gene F3) are expressed in a variety of cell types (7), whereas interferon consensus sequence binding protein, IRF-4 (PU.1 interacting protein), and IRF-7 are expressed predominantly in lymphoid cells (8, 9). IRF-5 and IRF-6 have not yet been characterized. Studies using the IRF-expressing cell lines and knockout mice reveal that each IRF family member exerts a distinct role in diverse biological processes, such as response to pathogens, cytokine signaling, cell growth regulation, or hematopoietic development (10–14). Although IRF-1 was first identified by its ability to bind the hexamer (AAGTGA) repeats in the IFNB promoter (15) and overexpression of IRF-1 is able to activate IFNA4 promoter in transient assay (16), the role of IRF-1 in virus-induced expression of type I IFN genes has been controversial, because virus-infected IRF-1-deficient mice or cells, expressed normal levels of IFNA and IFNB (17, 18). Mice with homozygous deletion of the p48 gene have shown a cell type-specific defect in type I IFN induction (19, 20); however, the role of p48 as a primary activator of type I IFN genes has also been questioned. In fact, mutant cells (U4A), defective in p48, are able to express IFN genes after virus infection. Therefore, we
have searched the ETS data base for a new IRF family members that may function as transcriptional activators of type I IFN genes and have identified IRF-3 (21) and, recently, a new variant of IRF-7, IRF-7H. In a transient expression assay, IRF-3 strongly synergizes with virus-mediated activation of IFNA and IFNB promoters (21, 22). Recent studies from several groups have shown that IRF-3 is phosphorylated at the carboxy-terminal serine cluster in infected cells and translocated to nucleus, whereby it interacts with the transcriptional coactivator, CBP/p300 (22–24). Overexpression of E1A that targets CBP/p300 inhibited IRF-3-mediated transactivation of IFNA promoter, thus providing additional evidence for the biological role of IRF-3-CBP/p300 interaction (25).

IRF-7 was first isolated as a protein binding to the QRE-2 present in the promoter region of (Qp) the Epstein-Barr virus-encoded nuclear antigen 1, and the cDNAs corresponding to three spliced variants of IRF-7 (A, B, and C) were identified (9). The levels of IRF-7 mRNA in various Epstein-Barr virus infected cells showed an inverse correlation with the activity of Qp promoter, and it was, therefore, suggested that IRF-7 may be a repressor of transcriptional activity of Qp. Whether IRF-7 indeed mediates repression of Qp activity remains controversial. Nonkwelo et al. (8) have identified IRF-1 and IRF-2 as a positive regulators of Qp, whereas IRF-7 alone had little effect on the activity of Qp promoter. In contrast, Zhang and Pagano (9) demonstrated that IRF-7 suppressed Qp activity and competed with the binding with IRF-1 to the interferon-sensitive response element in vitro (9).

The purpose of this study was to characterize the spliced variant IRF-7H and establish its role in the activation of IFN gene expression. We have analyzed the IRF-7H-mediated modulation of IFNA promoter activity. The expression of IRF-7, similar to the expression of IFNA genes, is limited to the cells of lymphoid origin. Overexpression of IRF-7H activates IFNA promoter and enhances virus-mediated stimulation of this promoter. Using deletion analysis, we have identified the presence of a transactivation domain located downstream of the DBD of IRF-7H. In addition, we have found that viral infection facilitates transfer of IRF-7H from cytoplasm to nucleus. Thus, the IRF-7H shares some of its functions with both IRF-1 and IRF-3 but shows a restricted expression.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—In transient transfection assay, 0.5 × 10⁶ fibroblasts or 5 × 10⁶ lymphoid cells were transfected with constant amount of DNA (5 μg) using Superfect transfection reagent (Qiagen), according to the manufacturer’s manual.

Plasmids—IRF-7H cDNA with entire open reading frame (ORF) was cloned into pSPORT and pCMV.SPORT (Life Technologies Inc.) at SalI to NotI site. Gal4/IRF-7H and its various carboxy-terminal deletion mutants were constructed by inserting the polymerase chain reaction amplified, BamHI –XhoI IRF-7H fragments into pSG424 vector as described previously (21). Gal4/IRF-7H (428–514) was created by removing the EcoRI fragment containing amino acids 1–427 of IRF-7H from the Gal4/IRF-7H (1–514) construct. All the IRF-7H deletion expression plasmids were created in a similar manner; 5’-primer containing the SalI restriction site and 3’-primer containing the XhoI site were used to generate desired IRF-7H fragments. All the deletion mutants used in this study were sequenced at the 5’- and 3’-end of the insert and contained an in-frame stop codon within the XhoI site. Mutant IFNA4/ CAT construct containing point mutation in the IRF binding site (IRF-E) was created by polymerase chain reaction-based mutagenesis; wild type IFNA4/CAT, IFNA6/CAT, and IRF-1 expression plasmids were described previously (2). IRF-7H-green fluorescent protein (GFP) expression plasmid was constructed by inserting the IRF-7H-XhoI-HindIII fragment into vector, pEGFP-N (CLONTECH Inc.)

Expression of GST-IRF-7 Fusion Protein and in Vitro Transcription and Translation—A full-length IRF-7H ORF was cloned in frame with the glutathione S-transferase gene in pGEX-4T-1 vector (Amersham Pharmacia Biotech) and transformed into BL21 bacteria. Purification of GST fusion was done as described previously (21). In vitro transcription and translation of IRF-7H was done in rabbit reticulocyte lysate according to the manufacturer’s manual.

Northern Blot Analysis and CAT Assay—Northern blotting of total RNA was done as described previously (26). IRF-7H cDNA encoding the entire ORF was used as a template for random-prime labeled probe. For CAT assay, transfected cells were collected, and RNA was isolated. An equal amount of protein from each transfection was used in the CAT assay as described previously (16). Each experiment was repeated three times.

Gel Mobility Shift Assay—A double-stranded oligonucleotide probe corresponding to IFNA4 3E (35 base pairs) was fill-in-labeled with [32P]dATP by Klenow polymerase. The binding reaction (25 μl) contained 5 ng of GST/IRF-7H fusion protein and was done as described previously (21). For the competition assay, an excess of unlabeled competitor oligonucleotide (IE and mutant IE) was added to the binding reaction 5 min prior to the addition of the probe.

RESULTS

Identification of a Novel cDNA Derived from Alternatively Spliced IRF-7 Transcript—In search of an IRF family member that participates in the regulation of IFNA gene expression, we screened the Human Genome Science Expressed Sequence Tag cDNA data base for homology to the DNA binding domain of IRF-1. Using the BLAST algorithms, a new IRF family member was identified and isolated. By comparing its sequence with the IRF-7 cDNA sequences deposited at NCBI GenBank™, this cDNA (referred as IRF-7H hereafter in this paper) was found to be a spliced variant of the IRF-7 gene. Examination of the Human Genome Science data base revealed that cDNA clones corresponding to the IRF-7H were observed in at least three libraries: a dendritic cell, a healing groin wound, and a keratinocyte library. This suggests that the IRF-7H is not generated by an aberrant splicing event. As shown in Fig. 1A, the IRF-7H cDNA (GenBank™ accession number AF076494) encodes a predicted protein of 514 amino acids that differs from IRF-7A by 18 amino acids at the very amino-terminal region (9). Like other IRF family members, IRF-7H contains five conserved tryptophan residues in the amino-terminal part of the protein.

The isolated IRF-7H cDNA sequence reveals the presence of two translation start codons (ATG) and in-frame stop codons upstream to the predicted ORF, which contains an imperfect Kozak sequence (27). Nevertheless, IRF-7H can be efficiently translated by in vitro transcription and translation and encodes a 67-kDa protein (Fig. 2B); the molecular mass of this protein confirms the predicted ORF. Therefore, it is likely that the IRF-7H transcripts are translated in vivo.

IRF-7 Is Predominantly Expressed in Lymphoid Cells and Can Be Induced by IFNA, Viral Infection, and LPS—To investigate the possible role of IRF-7, we first examined the tissue distribution of IRF-7 message and its relative level in response to various extracellular stimuli such as virus infection, IFN, and LPS. Using IRF-7H cDNA as a probe, this probe detects all the IRF-7 isoform messages. As shown in Fig. 2A, constitutive expression of IRF-7 gene can be predominantly detected in the tissues of the immune system, such as spleen, lymph node, thymus, and peripheral blood leukocytes (PBLs) (Fig. 2A). As previously reported (9), two IRF-7 transcripts of 2.6 and 2 kilobases were detected in all positive samples. The levels of the 2-kilobase transcript in PBLs were noticeable higher than those detected in other tissues. Although we do not know which transcript is equivalent to IRF-7H, the result of S1 mapping (data not shown) indicated that both IRF-7H and IRF-7A messages were expressed in equal abundance in human PBL and different lymphoid cell lines.

The expression of the IRF-7 gene can be induced by different extracellular stimuli. As shown in Fig. 2B, the relative levels of IRF-7 mRNA were increased in T-, B-, and monocyte cell lines treated with IFN-α, but not with IFN-γ, when compared with untreated cells (500 units/ml for 8 and 16 h). Interestingly, only
the 2-kilobase transcripts were detected in these cell lines, which exhibited distinct kinetics of induction. The expression of IRF-7 could also be induced by Newcastle disease virus (NDV) infection of human PBLs, and treatment with LPS (100 ng/ml for 6 h) had a similar effect (Fig. 2, C and D). No change in IRF-7H/IRF-7A ratio was observed upon virus infection or LPS treatment (data not shown). The protein synthesis inhibitor cycloheximide further augmented the stimulation in combination with IFNA and LPS. In contrast to lymphoid cells, NDV, IFNA, or poly(rI·rC) did not induce IRF-7 gene expression in human fibrosarcoma cells, 2FTGH (Fig. 2 D). Taken together, these results indicate that the expression of IRF-7, unlike IRF-3, is inducible by IFN and that the virus and induction can occur in the absence of protein synthesis in lymphoid cells.

IRF-7H Contains a Transactivation Domain Located between Amino Acids 120 and 237—Among the IRF family members, IRF-1 and IRF-3 were shown to be transactivators, whereas IRF-2 and interferon consensus sequence binding protein generally suppress the transcriptional activation. In order to determine the transcriptional potential of IRF-7H, full-length IRF-7H or its deletion mutant, without the putative DBD (amino acids 1–120), were cloned in frame with the GAL4 DNA binding domain (amino acids 1–147). The plasmids that express GAL4/IRF-7H fusion proteins were then cotransfected with CAT plasmid containing the thymidine kinase (TK) minimal promoter and four copies of GAL4 DNA binding site into Jurkat T cells. Both GAL4/IRF-7H and GAL4/IRF-7H DBD activated the GAL4/TK/CAT plasmid to a similar extent, indicating that IRF-7H contains a transactivation domain in its carboxyl-terminal half of the protein (data not shown). In order to map the transactivation domain of IRF-7H and examine the possibility that lymphoid tissue-specific cellular factors contributes to the transcriptional activity of IRF-7H, we tested a series of carboxyl-terminal deleted GAL4/IRF-7H DBD mutants to activate the reporter gene. As shown in Fig. 3 A, the transactivating potential of IRF-7H was significantly reduced when its carboxyl-terminal end (amino acids 418–514) was deleted. However, expression of the carboxyl-terminal end of IRF-7H (GAL4/IRF-7 428–514) alone did not confer transactivating activity. Interestingly, IRF-7H mutants with further truncation (amino acids 120–349 and 120–237) regained the ability to activate the reporter gene. These results indicate that transactivation domain of IRF-7H is likely located between amino acids 120 and 237 and that
deletion of the carboxyl-terminal region of IRF-7H may result in the conformational change of the protein and exposure of the transactivation domain. Because the sequence in the transactivation domain of IRF-7H is also present in the IRF-7A and IRF-7B, these two IRF-7 variants are expected to activate transcription as well.

**Overexpression of IRF-7H Activates the IFNA4 Promoter in a Transient Transfection Assay**—Because the expression of both IRF-7 and IFNA genes occurs predominantly in cells of the lymphoid origin, we next examined the role of IRF-7H in the activation of the IFNA promoters. Using a transient transfection assay, we assessed the transcriptional activity of murine IFNA4 promoter in L929 cells expressing a series of carboxyl-terminal truncated IRF-7 mutants fused with the GAL4 DNA-binding peptide. IRF-7-mediated activation of IFNA promoter. IFNA4/CAT activity was examined in L929 cells transfected with IRF-7 expression plasmid or its deletion mutants. An equal amount of DNA (5 μg) was used in each transfection; empty pCMV.S-PORT vector was used for the adjustment of DNA concentration. The GAL4/IRF-7 (428–514), designated RD (regulatory domain), was used as competitor of IRF-7-mediated activation of IFNA4 promoter with an increasing regulatory domain/IRF-7 ratio (1:1 and 2:1).

Overexpression of IRF-7H in IRF-1−/− mouse fibroblast in the presence and absence of NDV infection appeared to result in much higher level of activation of the IFNA promoter than in L929 cells (75-fold induction by IRF-7, 40-fold induction by NDV, and 285-fold induction by NDV and IRF-7) (Fig. 4B). However, this high level of activation is partially due to the low basal activity of the reporter gene in IRF-1−/− cells. Altogether, these results are in concurrence with the previous findings that NDV infection synergizes with IRF-3 in the activation of the IFNA promoter, thus identifying both IRF-3 and IRF-7H as a downstream target of the virus-activated signaling pathway. Furthermore, the finding that IRF-7H can highly activate IFNA4 promoter in the IRF-1−/− cells and that the presence of IRF-1 may actually interfere with the IRF-7-mediated activation of this promoter. Our recent preliminary data indicate the interaction between IRF-1 and IRF-7H.2

There are two observations supporting the notion that the IRF-7 activates the IFNA4 promoter via a direct binding to the IE of the IFNA4 promoter: 1) two point mutations (−94 and −103), in the IRF-E and AF1 binding sites (mutant IFNA4/CAT) of the IFNA4 promoter (−452) suppressed IRF-7-mediated activation by 80%. Importantly, these two mutations also exerted a comparable inhibitory effect on NDV-mediated activation (see Fig. 4B). 2) In the mobility gel shift assay (Fig. 4C), we detected specific binding of GST-IRF-7H to the IE. Whereas a 20-fold molar excess of wild type IE completely competed out the binding, the mutant IE, which contains the same mutations as mutant IFNA4/CAT, did not compete efficiently even at a 100-fold molar excess (Fig. 4C). The second band of slower mobility, seen upon binding of IRF-7H to IE, may result from the GST/IRF-7H homodimerization. We conclude from these experiments that activation of the IFNA4 promoter by IRF-7 is

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mediated through a direct binding of IRF-7 to the IE. It is noteworthy that we were unable to detect any binding of the recombinant IRF-1 to the IE probe under similar conditions, despite the fact that IRF-1 was shown to activate the IFNA promoter in transient transfection assay (16).

**NDV Infection Enhances Nuclear Localization of IRF-7H**—We and others have recently shown that phosphorylation of IRF-3 in infected cells facilitates its transport to the nucleus (22, 24). To determine whether viral infection also affects the cellular localization of IRF-7, we tagged IRF-7H with the GFP at the carboxyl terminus and analyzed its cellular localization in transfected L929 cells before and after infection with NDV. As a control, we analyzed and compared the intracellular localization of the GFP alone and IRF-3 tagged with GFP (24) in infected and uninfected cells. As shown in Fig. 5, expression of GFP was detected both in cytoplasm and nucleus 16 h after the transfection (t0) and 16 h post-NDV infection (t16). In contrast, IRF-7H/GFP fusion protein could be detected only in cytoplasm at 0 and 6 h postinfection (22 h after transfection). NDV infection induced translocation of IRF-7H/GFP into the nucleus. At 6 h postinfection IRF-7H/GFP was diffusely expressed in and around the nucleus. At 16 h postinfection, IRF-7H/GFP accumulated in the nucleus. However, at this time in uninfected cells, IRF-7H/GFP could also be deleted around and in the nucleus. The observed translocation of overexpressed IRF-7H/GFP to nucleus in the uninfected cells is consistent with our observation that overexpression of IRF-7 in L929 cells can activate IFNA reporter even in the absence of viral infection. Because we and others have previously shown that in infected cells, IRF-3 is also translocated into the nucleus (22, 23), we have examined whether overexpression of IRF-3 will modulate translocation of IRF-7H/GFP protein. When IRF-3 and IRF-7H/GFP expression plasmids were cotransfected, IRF-7H/GFP could be detected only in cytoplasm even in the infected cells (the cell shown in Fig. 5i contains two nuclei; therefore, the bright area represents the cytoplasm). These data indicate that overexpressed IRF-3 inhibits translocation of IRF-7 in infected cells. Interestingly, the IRF-3–5A mutant (24), which is unable to be phosphorylated, did not inhibit the nuclear transfer of IRF-7/GFP in infected cells (Fig. 5, i and h).

**Differential Activation of IFNA4 and IFNA6 Promoter by IRF-7H—NDV Infection of L929 cells induces transcriptional activity of IFNA4 but not IFNA6 genes promoter in transient expression assay. A similar difference in the expression of endogenous IFNA4 and IFNA6 was also detected (1). We had suggested that the distinct affinity of both the AF1-binding proteins and member(s) of the IRF family for the IFNA4IE and IFNA6IE might account for the differential expression of these two genes (2). To investigate whether activation of IFNA promoter by IRF family members shows the same specificity as NDV infection, we analyzed and compared the activation of IFNA4 and IFNA6 promoters by IRF-1, IRF-3, and IRF-7H in a transient transfection assay. The results show that whereas IRF-1 activated both promoters, overexpression of the IRF-3 and the IRF-7H in L929 cells activated only IFNA4 promoter but not (or poorly) the IFNA6 promoter (Fig. 6). These results indicate that IRF-3 and IRF-7H but not IRF-1 show the same specificity in the transcriptional activation of IFNA4 and IFNA6 promoter as NDV.

**Activation of IFNA Promoter by IRF-3 and IRF-7H Shows a Difference in Sensitivity to E1A**—It has been recently shown that viral infection triggers phosphorylation of IRF-3, which facilitates its association with CBP/p300 (22–24). The importance of the CBP/p300 and IRF-3 interaction for the activation of the IFNA genes is supported by the observation that E1A (128 and 138), which targets p300, suppresses the IRF-3- and NDV-induced activation of IFNA promoter, whereas a mutant E1A that lacks the p300-interacting domain does not (25). The IRF-7A was shown to be present in the same DNA-binding complex (VAF) with CBP/p300 on the interferon-stimulated
response element (28), we examined whether the IRF-7H-mediated activation of IFNA promoter can be modulated by E1A. Whereas in rat embryonic fibroblasts, expression of 13SE1A suppressed the IRF-1 and IRF-3-stimulated transcriptional activity (Fig. 7A), IRF-7H-mediated activation of IFNA promoter was not inhibited but enhanced. This result suggests that IRF-3 and IRF-7H function via nonoverlapping mechanisms. Interestingly enough, 12SE1A totally suppressed the IRF-1-, IRF-3-, and IRF-7-mediated transactivation both in rat embryonic fibroblast cells and L929 cells. A similar difference between the ability of 12S and 13SE1A in modulating the expression of HIV-LTR was observed previously (29). The mutant 12SE1A (12SE1A(Δp3000)), which is unable to bind p300, had no inhibitory effect on IRF-7H-mediated transactivation (Fig. 7B). We have previously shown that the carboxyl-terminal part of IRF-3 interacts with p300 when phosphorylated in infected cells (24). To examine whether CBP/p300 interaction also occurs in the carboxyl-terminal part of IRF-7H, we tested the effect of 12SE1A on mutant IRF-7H(237), which lacks the carboxyl-terminal region. The result showed that the 12SE1A suppressed both the IRF-7H- or IRF-7H(237) mutant-mediated activation of IFNA4 promoter. In contrast, 12SE1A(Δp3000) had no inhibitory effect.

**DISCUSSION**

In the present study, we have isolated a new, spliced variant of IRF-7, IRF-7H, and analyzed its potential role in the transcriptional activation of IFNA genes. Expression of IRF-7 can be stimulated by both viral infection and IFNA in the absence of protein synthesis, indicating that IRF-7 is the immediate...
early gene responding to these signals. The specific induction of IRF-7 gene expression by type I IFN, but not by IFN-γ, is of interest in view of the fact that IRF-1 is induced much more efficiently by IFN-γ than by IFNA and expression of IRF-3 is not stimulated by IFNs or virus. These data indicate that in the context of viral infection, the expression of these IRFs shows distinct specificity.

The data presented in this study identified IRF-7 as a transcriptional activator, which is in concurrence with the recent results of Wathlet et al. (28). Using the Gal4-IRF-7H fusion protein and carboxyl-terminal deletion mutants of IRF-7H, we have identified the transactivation domain of IRF-7 to be located between amino acids 120 and 237. The transactivation potential of IRF-7 is apparently independent of the target gene because a similar degree of activation was obtained with both Gal4 and IFNA reporter plasmids.

Using deletion analysis and in vivo competition assay, we have identified an additional regulatory domain in the carboxyl-terminal end of IRF-7H protein (amino acids 428–514) that acts as a dominant negative mutant capable of suppressing the IRF-7H-mediated transactivation. This domain, which is present in the IRF-7A and IRF-7B, but missing in IRF-7C, contains a serine-rich region (Fig. 1A) and a few imperfect NES-like motifs. Despite the fact that exon skipping of IRF-1 mRNA was suggested as one of the mechanisms by which the IRF-1 gene is inactivated in human leukemia (30), the identification of multiple variants of IRF-7 (possibly derived from the differential splicing) is unique to the IRF-7 gene and has not yet been demonstrated in the other IRFs. It is likely that some of these IRF-7 spliced variants may function as dominant negative mutants; as such, an unbalanced expression of these variants may be of biological importance.

We have previously shown that virus-stimulated phosphorylation of IRF-3 on serines (396, 398, 402, and 405) and tyrosine (404) is critical for the translocation of IRF-3 into the nucleus and its interaction with a transcriptional cofactor, CBP/p300 (24). In contrast, Yoneyama et al. (22) attributed a critical role to serines 385 and 386 (22). Although the serine-rich region of IRF-7H could potentially be phosphorylated, the observation that IRF-7H mutants (IRF-7 349 and 237) that had this region deleted were still able to stimulate transcription of IFNA promoter in a transient transfection assay suggests that phosphorylation of this cluster does not play a limiting role in the transactivating ability of IRF-7H. These phosphorylation sites, however, seem to be required for the observed synergy between virus and IRF-7H in the activation of the IFNA promoter, which was observed only with the full-length IRF-7H but not with its carboxyl-terminal deletion mutants. Similar to the observation with IRF-3, the transport of IRF-7H into the nucleus was facilitated by viral infection, but when overexpressed, IRF-7H could also be found in the nucleus of unin-

**Fig. 6.** Specific activation of IFNA4 and IFNA6 promoters by NDV infection and overexpression of IRF-3 or IRF-7. Transfection and infection were performed as described under “Experimental Procedures.” IFNA4 and IFNA6 containing 152 and 145 base pairs of the respective promoter regions were used. The transfection efficiency was normalized with the β-galactosidase activity.

**Fig. 7.** E1A modulates IRF-7-mediated activation of IFNA promoter. A, transfection in rat embryonic fibroblast (REF) cells. IFNA4/CAT (2 μg) and the indicated IRF expression plasmid (1.5 μg) were co-transfected with or without the plasmid (1.5 μg) encoding either 12SE1A or 13SE1A. The CAT activity of each transfection was determined from sample collected 48 h after the transfection. The expression of 13SE1A exhibits the opposite effect on IRF-3- and IRF-7-mediated activation of IFNA promoter. B, the effect of overexpression of 12SE1A or its mutant, 12SE1AΔp300, on the IRF-7-mediated activation of IFNA promoter was examined in L929 cells. Mutant IRF-7(237) was described previously (see Fig. 3). The experiment was done under the same conditions as in A. The values represent the mean of two separate experiments.
fected cells. Because both IRF-7 and IRF-3 were found as components of the VAF complex binding to the interferon-stimulated response element, it was suggested that IRF-3 and IRF-7 are associated in cells (28). We expected, therefore, that the translocation of IRF-7 into the nucleus might be facilitated in the presence of IRF-3. Surprisingly, however, we found that overexpression of IRF-3 blocked translocation of IRF-7 in infected cells, indicating that these two factors are not transported to the nucleus as a heterodimer complex. These results rather suggest that, when overexpressed, IRF-3 may serve as a competitor for the virus-induced modifications and thus inhibit nuclear translocation of IRF-7. This assumption is further supported by the finding that the mutant, IRF-3(5A) is not inhibitory.

The interaction of transcriptional factors assembled on the IFNB promoter with CBP/p300, including IRF-1, IRF-3, and IRF-7, was suggested to be required for inducible expression of the IFNβ gene (28). Although the direct binding of IRF-7 to CBP/p300 has not been demonstrated, the presence of IRF-3, IRF-7, and CBP/p300 was detected in the VAF complex associated with the interferon-stimulated response element (28). The fact that the IRF-7-mediated transactivation of IFNβ promoter region is inhibited by 12SE1A but not by its mutant that is unable to bind CBP/p300 suggests that interaction of IRF-7 with CBP/p300 is required for its transcriptional activity. Interestingly, whereas the activation of IFNβ promoter by all these three IRFs is inhibited by 12S/E1A, 13S/E1A only inhibits its IRF-1 and IRF-3-mediated activation but stimulates the IRF-7 activation. Both of these forms of E1A can bind CBP/p300, but only 13SE1A contains the CR3 domain that interacts with TBP and TAF (29). Thus, the distinct repression and activation of IRF-3 and IRF-7, respectively, by 13S/E1A may reflect a subtle difference in transcriptional cofactors with which IRF-7 and IRF-3 interact. Differential modulation of HIV-LTR activity by 13S and 12SE1A has previously been reported, and it was shown that activation and repression of HIV-LTR activity by 12S and 13SE1A, respectively, is mediated by different sets of transcriptional factors (29).

Although the transcriptional complex-enhancerosome that binds to the promoter of IFNA genes has not yet been identified, several observations indicate that IRF-7 plays a critical role in the induction of IFNA genes. First, inducibility of the IFNA genes and expression of IRF-7 show the same cell type specificity, both being preferentially expressed in cells of the lymphoid origin. Second, overexpression of IRF-7 exhibits the same specificity in the transcriptional induction of IFNA4 and IFNA6 promoters as virus infection. Third, IRF-7 specifically binds the IE of IFNA4 promoter in vitro. Because many of the functional characteristics of IRF-7 are similar to those of IRF-3, it may be possible that a cooperative interaction of both of these factors is necessary for the induction of IFNA gene transcription. Indeed, while this manuscript was being prepared, IRF-3 and IRF-7, but not IRF-1, were found to be components of the enhancerosome associating with the promoter of IFNB gene (29). Further studies are in progress to determine whether the expression of IFNA genes is determined by the cell-specific interaction between the members of the IRF family or by their interaction with lymphoid cell-specific transcription factors.

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REFERENCES

1. Hisamatsu, H., and Bisat, F., Raj, N. B., and Pitha, P. M. (1988) Nucleic Acids Res. 16, 6067–6083.
2. Au, W. C., Su, Y., Raj, N. B., and Pitha, P. M. (1993) J. Biol. Chem. 268, 24032–24040.
3. Raj, N. B., and Pitha, P. M. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 7426–7430.
4. Raj, N. B., Au, W. C., and Pitha, P. M. (1994) J. Biol. Chem. 269, 11360–11365.
5. Braganza, J., Genin, P., Bandu, M. T., Darraq, N., Vignal, M., Causer, C., Doly, J., and Civas, A. (1997) J. Biol. Chem. 272, 22154–22162.
6. Veala, S. A., Schindler, C., Leonard, D., Fu, X. Y., Aebbersold, R., Darnell, J. E., Jr., and Levy, D. E. (1992) Mol. Cell. Biol. 12, 3315–3324.
7. Nguyen, H., Hiscott, J., and Pitha, M. P. (1997) Cytokine and Growth Factor Reviews 8, 293–312.
8. Nonkelois, C., Raf, I. K., and Sample, J. (1997) J. Virol. 71, 6887–6897.
9. Zhang, L., and Pagano, M. S. (1997) Mol. Cell. Biol. 17, 5749–5757.
10. Holtschke, T., Lohler, J., Kanno, Y., Fehr, T., Giese, N., Rosenbauer, F., Lou, J., Knebelob, K. P., Gabriele, L., Waring, J. F., Bachmann, M. F., Zinkernagel, R. M., Morse, H. C., III, Ozato, K., and Horak, I. (1996) Cell 83, 307–317.
11. Mittrucker, H. W., Matsuyama, T., Grossman, A., Kurig, T. M., Potter, J., Shahinian, A., Wakeham, A., Patterson, B., Ohashi, P. S., and Mak, T. W. (1997) Science 275, 540–543.
12. Tamura, T., Ishihara, M., Lammhi, M. S., Tanaka, N., Oishi, I., Aizawa, S., Matsuyama, T., Mak, T. W., Taki, S., and Taniguchi, T. (1997) Leukemia 11, Suppl. 3, 439–440.
13. Salkowski, C. A., Barber, S. A., Detore, G. R., and Vogel, S. N. (1996) J. Immunol. 156, 3107–3110.
14. Duncan, G. S., Mittrucker, H. W., Kagi, D., Matsuyama, T., and Mak, T. W. (1996) J. Exp. Med. 184, 2043–2048.
15. Fuji, T., Shibuya, J., Hoita, I., Yamanishi, K., and Taniguchi, T. (1987) Cell 49, 357–367.
16. Au, W. C., Raj, N. B., Pine, R., and Pitha, P. M. (1992) Nucleic Acids Res. 20, 2877–2884.
17. Reis, L. F., Ruffner, H., Stark, G., Aget, M., and Weissmann, C. (1994) EMBO J. 13, 4798–4806.
18. Ruffner, H., Reis, L. F., Naf, D., and Weissmann, C. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 11503–11507.
19. Kimura, T., Kadokawa, Y., Harada, H., Matsumoto, M., Sato, M., Kashiwazaki, Y., Tarutani, M., Tan, R. S., Takasugi, T., Matsuyama, T., Mak, T. W., Noguchi, S., and Taniguchi, T. (1996) Genes Cells 1, 115–124.
20. Kawakami, T., Matsumoto, M., Sato, M., Harada, H., Taniguchi, T., and Kitagawa, M. (1995) FEBS Lett. 358, 225–229.
21. Au, W. C., Moore, P. A., Lowther, W., Juang, Y. T., and Pitha, P. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 11657–11661.
22. Yoneyama, M., Suhara, W., Fukuhara, Y., Fukuda, M., Nishida, E., and Fujita, T. (1998) EMBO J. 17, 1087–1095.
23. Weaver, B. K., Kumar, K. P., and Reich, N. C. (1996) Mol. Cell. Biol. 18, 1559–1566.
24. Lin, R., Heybroek, C., Pitha, M. P., and Hiscott, J. (1998) Mol. Cell. Biol. 18, 2986–2996.
25. Jiang, Y.-T., Lowther, W., Kellum, M., Au, W.-C., Lin, R., Hiscott, J., and Pitha, P. M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 9837–9842.
26. Raj, N. B., Engelhardt, J., Au, W. C., Levy, D. E., and Pitha, P. M. (1998) J. Biol. Chem. 273, 16688–16696.
27. Kozak, M. (1989) J. Cell Biol. 124, 229–241.
28. Wathlet, M. G., Lin, C. H., Parekh, B. S., Ronco, L. V., Howley, P. M., and Maniatis, T. (1998) Mol. Cell. Biol. 18, 507–518.
29. Parker, S. F., Felzen, L. R., Perkins, N. D., Imperiale, M. J., and Nabel, G. J. (1997) J. Virol. 71, 2004–2012.
30. Harada, H., Kudo, T., Ogawa, T., Tamura, T., Kitagawa, M., Tanaka, N., Lammhi, M. S., Taniguchi, T. (1994) Oncogene 9, 3313–3320.