IRF-7 plays an essential role in virus-activated transcription of IFNA genes. To analyze functional domains of IRF-7 we have constructed an amino-terminal deletion mutant of IRF-7 (237–514) which exerted a dominant negative (DN) effect on virus-induced expression of the endogenous Type I IFN genes. Focusing on the molecular mechanism underlying the dominant negative effect of IRF-7 DN, we found that virus-activated transcription of endogenous IFNA genes requires full-length IRF-7 and that Serine 463 and 484 play an essential role. While IRF-7 DN had no effect on virus-stimulated nuclear translocation of IRF-3 and IRF-7, the binding of IRF-7 DN to IRF-3 and IRF-7 was detected by GST pull-down assay as well as by immunoprecipitation in infected cells, indicating that IRF-7 DN targets both IRF-7 and IRF-3. The region by which IRF-7 interacts with IRF-3 was mapped between amino acid 418 and 473. Overexpression of IRF-7 DN in virus-infected 2FTGH cells resulted in an inhibition of IFN synthesis and in a significant reduction of binding of both IRF-3 and IRF-7 to the IFNA1 promoter. Interestingly, the IRF-7 DN-mediated suppression of IFNA gene expression can be negated by overexpression of IRF-3. Altogether these results suggest that the IRF-3/IRF-7 complexes are biologically active and are involved in virus-activated transcription of endogenous IFNA genes.

**Key Words:** interferon; interferon regulatory factor-7; interferon regulatory factor-3; dominant negative; transcription; New Castle Disease Virus; Sendai Virus.

**INTRODUCTION**

Type I IFNs (IFNA and IFNB) exert multiple biological activities upon binding to their cognate cellular receptors. The Type I IFN-activated Jak-STAT signaling pathway leads to the expression of a set of immediate-early response genes (IFN stimulated genes, ISGs), which play important roles in innate and adaptive immune response against viral and bacterial infection (Darnell, 1997; Stark et al., 1998). The inducible expression of Type I IFN gene (IFNA and IFNB) is tightly regulated at the transcriptional level (Bisat et al., 1988). While the synthesis of IFNA is mainly restricted to the cells of lymphoid origin, the synthesis of IFNB can be induced in most of the cell types. Recently, a CD4+CD11c+ type 2 dendritic cell precursor has been identified as the high IFNA producing cell that, upon viral infection, produces 200-1000 fold more Type I IFN than do the other lymphoid cell types (Siegal et al., 1999).

Extensive studies focusing on the regulation of virus-induced transcription of a single-copy IFNB gene have revealed that it requires the assembly of a multicomponent complex, named enhanceosome, on the promoter.

**Analysis of Functional Domains of Interferon Regulatory Factor 7 and Its Association with IRF-3**

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This enhanceosome consists of NFKB, IRFs, ATF2, c Jun, HMGI (Y), and transcription coactivator, p300/CBP (Kim and Maniatis, 1997; Merika et al., 1998; Wathelet et al., 1998). The p300/CBP has been shown to acetylate the local histone H3 and H4 and possibly make the chromatin structure more accessible to the transcription machinery (Agalioti et al., 2000; Parekh and Maniatis, 1999). In contrast, the factors involved in the regulation of IFNA genes are much less defined. Although the promoter region containing the virus-responsive element (VRE) of all IFNA genes show only small variations, the expression of individual IFNA subtype varies in a cell type specific manner (Bisat et al., 1988).

The promoters of IFNA, IFNB, and ISG genes all contain multiple GAAANN repeats that constitute the binding sites (IRF-E, PRD-II, and ISRE, respectively) for proteins of the IRF family (Nguyen et al., 1997). The IRF family members share high homology in their N-terminal DNA-binding domain (DBD) which contains five conserved tryptophan repeats and is related to Myb (Veals et al., 1992), while the C-terminal regions are more diverse and are related to the proteins of the Smad family (Eroshkin and Mushegian, 1999). All the cellular IRFs bind to similar DNA sequence. The structural analysis of IRF-2 bound to the DNA has shown that the recognition sequence is AANNGAAA (where N can be any base; Fujii et al., 1999).

Recent studies indicated that the activation of IRF-3

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and IRF-7 are important for the expression of Type I IFN genes (Au et al., 1995; Lin et al., 1998; Navarro et al., 1998; Weaver et al., 1998; Yoneyama et al., 1998). It was shown that in infected cells, IRF-3 is phosphorylated on the serine residues close to the C terminus of IRF-3 and accumulates in the nucleus, where it interacts with CBP/p300 (Kumar et al., 2000; Lin et al., 1998; Weaver et al., 1998; Yoneyama et al., 1998). IRF-7 was also shown to participate in the regulation of Type I IFN gene expression in infected cells. (Au et al., 1998; Sato et al., 1998) and four splice variants of human IRF-7 (IRF-7A, B, C, and H) have been identified (Au et al., 1998; Zhang and Pagano, 1997). Although IRF-7A, B, and C were initially demonstrated as negative regulators of the Qp region that regulates expression of ENBA-1 gene in Type III latency of EB virus infection (Zhang and Pagano, 1997). We, as well as others, have shown that overexpression of IRF-7H or IRF-7A result in an activation of promoters of Type I IFN genes (Au et al., 1998; Wathelet et al., 1998). Unlike IRF-3, which is constitutively expressed, the expression of IRF-7 is inducible by Type I IFN, and by the EB virus-encoded latent membrane protein 1 (LMP-1) (Au et al., 1998; Marie et al., 1998; Sato et al., 1998; Zhang and Pagano, 2000). The importance of murine IRF-7 in the Type I IFN gene expression was first demonstrated by Taniguchi’s group, who showed that virus-activated transcription of Type I IFN genes depends on synthesis of IRF-7 involved in the positive feedback regulation of Type I IFN genes (Sato et al., 1998). Levy’s group has then shown that virus infection leads to transcriptional activation of the IFNB and IFNA4 genes, while the expression of the other IFNA genes requires interferon-mediated induction of IRF-7 (Marie et al., 1998). We have shown recently that induction of all the human IFNA subtypes by viral infection requires IRF-7, such that in cell line 2FTGH that is incapable of expressing IRF-7 gene (Au et al., 1998; Lu et al., 2000), ectopic expression of IRF-7H (hereafter referred as IRF-7) restores virus-induced expression of IFNA genes (Yeow et al., 2000). These results suggest that tissue-specific expression of IFNA genes is in part regulated by the presence of IRF-7.

In this study, we have used a dominant negative mutant of IRF-7 to dissect the molecular interactions that are essential for the virus-activated expression of endogenous IFNA genes. Our results revealed the functional importance of the interaction between IRF-3 and IRF-7 in IFNA gene expression; furthermore we have mapped the association domains through which IRF-3 and IRF-7 interact.

RESULTS

The carboxyl-terminal part of IRF-7 (237–514) suppresses virus-mediated activation of murine IFNA and IFNB genes

We have shown previously that the amino-terminal portion of IRF 7 (1–237) contains a DNA binding domain and a transactivation domain, whereas the carboxyl terminus of IRF-7 (428–514) designated as a regulatory domain (RD) is inhibitory to the virus-activated expression of murine IFNA4 reporter gene (Au et al., 1998). Similarly, an auto inhibitory domain has also been located at the C-terminal part of IRF-3 (380–427; Lin et al., 1999). It was demonstrated that IRF-4 and IRF-8 (ICSBP) contained an IRF association domain (IAD) which mediated their interaction with IRF-1, IRF-2, and proteins of ets family, such as PU.1 (Meraro et al., 1999; Nguyen et al., 1997). Protein sequence analysis indicates that IRF-3 and IRF-7 also contain regions that show significant homology to the IAD (Meraro et al., 1999; also see Fig. 6B). To better understand the molecular interactions that may be critical for the ability of IRF-7 to bind DNA as well as to modulate transcription of IFNA genes, we generated a flag-tagged IRF-7 deletion mutant consisting of a.a. 237–514 of IRF-7. This IRF-7 mutant, which contains neither the DBD nor the transactivation domain, was tested for its ability to modulate transcription of murine Type I IFN genes in L929 cell line that constitutively expresses IRF-7 dominant negative(DN) (237–514). The virus-induced expression of endogenous IFNA and IFNB genes was determined by semiquantitative RT-PCR. No expression of IFNA and IFNβ messages was detected in infected IRF-7 DN (237–514) expressing cells, while in infected parental cells, both IFNA and IFNB in RNA were detected (Fig. 1). The β actin and IRF-7 DN (237–514) messages were detected in all samples. These cells also expressed both IRF-3 and IRF-7 (data not shown). It was shown previously that IRF-3 alone can activate expression of murine and human IFNB genes (Marie et al., 1998; Yeow et al., 2000). The absence of both IFNA and IFNB mRNAs in infected, IRF-7 DN (237–514) expressing L-929 cells indicates that IRF-7 DN (237–514) may target both IRF-7 and IRF-3.

Virus infection leads to a phosphorylation of IRF-3 at the C-terminal serine-rich region (Lin et al., 1998; Weaver et al., 1998) and phosphorylation of S385 and S386 is required for the translocation of IRF-3 into the nucleus and its interaction with p300/CBP (Yoneyama et al., 1998). Furthermore, phosphorylation at the downstreamed serine and threonine residues was shown to induce a conformational change, releasing the autoinhibitory (AI) domain and facilitating the DNA-binding of IRF-3 (Lin et al., 1999). Although the S425 and S426 residues of murine IRF-7 were also found to be important to its transcriptional activity and homodimer formation (Marie et al., 1998, 2000), the biological role of these two serine residues in human IRF-7 have never been determined. Since IRF-7 DN (237–514) contains the consensus serine-rich sequence, we sought to determine whether IRF-7 DN (237–514) is phosphorylated in infected cells and whether the S483 and S484 of human IRF-7 are targeted for phosphorylation. To this effect, we created IRF-7 DN (237–514) phosphorylation mutant (IRF-7 DN ph-) in which serine residues

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at the position 483 and 484 were replaced by phenylalanine and alanine, respectively. The plasmid encoding IRF-7 DN (237–514) or IRF-7 DN ph- was transfected to 293 cells and 16 h posttransfection; cells were labeled with $^{32}\text{P}$-orthophosphate and infected with Sendai virus for 4 h. As shown in Fig. 2, Sendai virus infection of IRF-7 DN (237–514) transfected 293 cells resulted in the phosphorylation of IRF-7 DN (237–514), while no phosphorylation of IRF-7 DN ph- was detected. It is noteworthy that the IRF-7 DN (237–514) was also phosphorylated at low levels in uninfected cells, which indicates that a constitutive kinase activity is present in uninfected cells. The relative levels of IRF-7 DN protein were determined by the Western blot in which IRF-7 DN (237–514) and IRF-7 DN ph- mutants were expressed in transfected cells at about the same level.

IRF-7 DN (237–514) does not inhibit the virus-induced nuclear translocation of IRF-3 and IRF-7

It was suggested that IRF-3 and IRF-7 may be targeted by a similar kinase (Marie et al., 1998). To further dissect the mechanism by which IRF-7 DN (237–514) suppressed the virus-induced IFNA gene expression, we tested whether IRF-7 DN (237–514) could compete with IRF-3 and IRF-7 for the virus-activated kinase and consequently prevent the nuclear localization of IRF-3 and IRF-7 in virus-infected cells. As shown in Fig. 3, IRF-7/GFP and IRF-3/GFP are mainly localized in the cytoplasm of uninfected 2FTGH cells, and upon Sendai virus infection, both IRF-7/GFP and IRF-3/GFP are concentrated in the nucleus. The virus-induced nuclear translocation of IRF-7/GFP and IRF-3/GFP was not affected by the presence of IRF-7 DN (237–514). Therefore, the inhibition conferred by IRF-7 DN (237–514) is not due to the inhibition of the nuclear translocation of IRF-3 or IRF-7. It is noticeable that the distribution of IRF-7/GFP in Sendai virus infected 2FTGH cells is more distinct than that observed previously in NDV-infected L-929 cells (Au et
al., 1998); this difference could be due to the different cell types and viruses used in the experiments.

Activation of endogenous IFNA genes in virus-infected human 2FTGH cells requires the presence of full-length IRF-7 and its phosphorylation

We have previously shown that IRF-7 has a critical role in activation of IFNA genes in human 2FTGH cells (Yeow et al., 2000). Using these cells, we analyzed the functional domain of IRF-7 required for the transcriptional activation of the endogenous IFNA genes. The IRF-7 deletion mutants were therefore expressed in 2FTGH cells and the virus-mediated expression of IFNA and IFNB were described under Materials and Methods. Two sets of primers were used to amplify IRF-7 transcripts, in which a primer set (5′ primer ATGCCAGTCCCCGAGGCCCTG and 3′ primer GTACACCTTGTGCGGGTC) specific for the 5′ end of IRF-7 was used to detect the transcripts of IRF-7 (1–237) and IRF-7 (1–349). For the detection of IRF-7 transcript in the other transfected cells, a primer set specific for the 3′ end of IRF-7 was used as described under Materials and Methods. (C) A summary of the result from Fig. 3B. The + or − is given based on the presence or absence of amplified DNA fragments detected by the ethidium bromide staining. ND, not done.

FIG. 4. Activation of expression of endogenous IFNA genes by virus requires an intact IRF-7 protein. (A) Conditions for the semiquantitative RT-PCR. Various amounts of total RNA collected from Sendai virus-infected, IRF-7 expressing 2FTGH cells: 0.33 μg (lane 1), 1 μg (lane 2), or 3.3 μg (lane 3) was used for RT-PCR (30 cycles) to amplify IFNA messages. cDNA derived from 1 μg of RNA was used for PCR by 25 cycles (lane 4), 30 cycles (lane 5), or 35 cycles (lane 6) of amplification. One microgram of RNA and 30 cycles of amplification were used for all the RT-PCR performed in Figs. 1, 3B, and 6. (B) 2FTGH cells were transfected with the indicated plasmids and 16 h posttransfection, cells were infected with Sendai virus for 6 h. The levels of respective transcripts were determined by RT-PCR. The primer set used to amplify the 3′ part of the IRF-3 mRNA cannot detect the expression IRF-3/p65. The primer set used to amplify IFNA and IFNB were described under Materials and Methods. Two sets of primers were used to amplify IRF-7 transcripts, in which a primer set (5′ primer ATGCCAGTCCCCGAGGCCCTG and 3′ primer GTACACCTTGTGCGGGTC) specific for the 5′ end of IRF-7 was used to detect the transcripts of IRF-7 (1–237) and IRF-7 (1–349). For the detection of IRF-7 transcript in the other transfected cells, a primer set specific for the 3′ end of IRF-7 was used as described under Materials and Methods. (C) A summary of the result from Fig. 3B. The + or − is given based on the presence or absence of amplified DNA fragments detected by the ethidium bromide staining. ND, not done.
IRF-7 DN (237–514)-mediated activity and that transcriptional activation of endogenous IFNA, genes unlike transient transfection (Au et al., 1998), requires full-length IRF-7.

Interestingly, expression of IRF-7 mutants, IRF-7 (1–237) and IRF-7 (1–349), suppressed the virus-induced IFNB expression. Consistent with this result, the synthesis of biological active Type I IFN (IFNB) from these cells (50 U/ml) was about 15% of the input was then used for the GST pull-down assay. Binding of in vitro translated IRF-7 proteins to immobilized GST/IRF-3 fusion protein is shown in the middle panel. No binding to GST alone was detected. Binding of in vitro translated IRF-7 FL to the C-terminal part of GST/IRF-7 (300–514) is shown in the right panel. The percentage of retention as measured by the phosphorimager is given. The amino acid sequence of the C-terminal domain of IRF-7 (418–473) which interacts with IRF-3 is shown as well as the alignment of corresponding region of IRF-3 and IRF-8 (ICSBP). Asterisk marks the conserved leucine (L) and glycine (G) residues that were shown to be important for the interaction between IRF-8 and IRF-1 or IRF-2.

Since IRF-3 and IRF-7 are both targeted by IRF-7 DN (237–514), it is conceivable that the activity of IRF-7 DN (237–514) is inversely correlated with the relative level of IRF-3 and IRF-7.

Determination of the interaction between IRF-7 and IRF-3

Since IRF-7 DN (237–514) exhibited inhibitory effect on virus-induced expression of IFNA and IFNB genes, we hypothesized that the mechanism underlying the IRF-7 DN (237–514) activity may be through disrupting the interaction between IRF-3 and IRF-7. We, therefore, used GST pull-down assay to measure the binding of in vitro labeled IRF-7 and its deletion mutants to the immobilized GST/IRF-3 fusion protein. As shown in Fig. 5A, while neither IRF-7 nor its deletion mutants bind GST alone, full-length IRF-7 (1–514) and its deletion mutants IRF-7
(1–237) and (237–514) bind to full-length IRF-3 with comparable affinity as determined by the percentage of retention. Only the IRF-7 mutant (237–418) did not bind IRF-3. To map the carboxyl terminal region of IRF-7 that interacts with IRF-3, we analyzed the binding of IRF-7 IAD (239–473) and IRF-7 RD (418–514) to GST/IRF-3. The result showed that both of these proteins can bind IRF-3 (Figs. 5A and 5B). These results indicate that IRF-7 is able to bind to IRF-3 through two regions: one is located within the N-terminal half (1–237) of the protein and the other is located between a.a. 418 and 473. Since IRF-7 DN (237–514) is missing in the N-terminal part of IRF-7, we have not characterized in details this binding site. Interestingly, we have also found that IRF-7 can dimerize with itself through the C-terminal region (300–514) (Fig. 5A) and with IRF-7 DN (data not shown). The region of IRF-7 (237–418) which is incapable of binding to the IRF-3 (Figs. 5A and 5B) did not exhibit a dominant negative effect on the expression of endogenous IFNA genes (Fig. 4C). To further confirm this finding, the synthesis of biological active Type I IFN from these cells was determined. In agreement with the RT-PCR result, the IFN titer in IRF-7 DN (237–514)-transfected cells (90 U/ml) is significantly lower than that in IRF-7 (237–418)-transfected cells (282 U/ml). These results support the idea that IRF-7 DN (237–514)-mediated effect is derived from a direct interaction between IRF-7 DN (237–514) and IRF-3 and IRF-7.

To map the domain of IRF-3 that interacts with IRF-7, we examined the binding of deletion mutants of IRF-3 expressed as GST fusion proteins (Fig. 6A) to the in vitro translated full-length IRF-7. IRF-7 did not bind the N-terminal half (1–231) of IRF-3 and the interaction domain in IRF-3 was mapped to a.a 306–427; this region overlaps with the IRF-3 IAD (199–375) (Fig. 6A).

To determine whether the interaction between IRF-3 and IRF-7 DN (237–514) can also occur in vivo, the lysate from 2FTGH cells transfected with IRF-3 expression plasmid and Flag-tagged IRF-7 DN (237–514) or Flag-tagged IRF-7 DN ph- were immunoprecipitated with anti-Flag antibody. The immunoprecipitated proteins were resolved by SDS–PAGE and the presence of IRF-3 was determined by Western blot. As shown in Fig. 6B, the coprecipitation of IRF-7 DN (237–514) with IRF-3 could be detected only in the infected cells; in contrast, very little binding of IRF-7 DN ph- to IRF-3 was observed in these cells. These result suggest that virus-induced phosphorylation may facilitate the interaction between IRF-3 and IRF-7 DN (237–514) in vivo.

Overexpression of IRF-7 DN (237–514) decreases the binding of IRF-3 and IRF-7 to IFNA1 VRE in infected cells

We have shown previously that both IRF-7 and IRF-3 can bind to the VRE of IFNA1 promoter (Yeow et al., 2000). We have therefore examined whether overexpression of IRF-7 DN (237–514) in the transfected cells will affect binding of these two IRFs to this VRE. The binding was measured using the DNA pull-down assay in which the oligonucleotides corresponding to the VRE of IFNA1 promoter, immobilized to magnetic beads, were incubated with whole-cell lysates from uninfected and infected 2FTGH cells, overexpressing IRF-7 alone or IRF-7 and IRF-7 DN (237–514). As shown in Fig. 7, IRF-3 from infected cells but not from uninfected cells binds to IFNA1 VRE and no binding to the beads only was detected (lane indicated by an arrow). When IRF-7 DN (237–514) was expressed in these cells, binding of IRF-3 to the IFNA1 VRE was significantly reduced. The reduction of IRF-3 binding was not a result of lower levels of
IRF-3 protein in these cells, since the levels of IRF-3 determined by Western blot were comparable in all cell lysates examined. Since the efficiency of transfection of IRF-7 DN (237–514) in 2FTGH cells in these experiments is about 70%, the residual IRF-3 binding activity (compare lane 2 and 4) may be derived from the IRF-3 in untransfected cells. Similarly, binding of IRF-7 was also decreased in the presence of IRF-7 DN (237–514). However, it is noticeable that IRF-7 can bind to the IFNA1 VRE at low levels even in the absence of virus infection. The ability of IRF-7 DN (237–514) to decrease the binding of IRF-3 and IRF-7 to IFNA1 VRE suggests that the DNA-binding affinity IRF-3/IRF-7 DN dimer which contains only one DNA-binding domain is not sufficient for stable binding to IFNA1 VRE. Taken together, the reduction of binding of IRF-3 and IRF-7 correlates with the observed suppression of endogenous IFNA gene expression by IRF-7 DN (237–514), thus suggesting that IRF-7 DN targets both IRF-7 and IRF-3 functions.

**DISCUSSION**

It was shown that IRF-7 is required for the expression of IFNA genes in infected human and mouse cells (Yeow et al., 2000). To better understand the molecular interaction between IRF-7 and the other components of the IFNA-specific transcription complex, we have studied the mechanism by which IRF-7 DN (237–514) inhibited the virus-induced expression of endogenous IFNA and IFNB genes. Since IRF-7 DN (237–514) does not contain DBD, the inhibitory effect is likely a result of an interference of the protein–protein interactions that are essential for the virus-induced IFNA gene expression. It was shown previously that both IRF-3 and IRF-7 participate in Sendai virus-mediated induction of IFNB gene (Whateleff et al., 1998); however, IFNB gene can be induced in the absence of IRF-7 (Yeow et al., 2000). The ability of IRF-7 DN (237–514) to inhibit expression of IFNB gene in the absence of IRF-7 indicates that IRF-7 DN (237–514) targets not only IRF-7 but also IRF-3.

We have shown that IRF-7 DN (237–514) is efficiently phosphorylated upon virus infection and that S483 and S484 of IRF-7 are critical both for the IRF-7-mediated activation of endogenous IFNA genes and for the IRF-7 DN (237–514)-mediated inhibitory effect. Since no phosphorylation was detected in IRF-7 DN in which S483A and S484F were changed to an alanine or phenylalanine, this result suggested that the other serine and threonine residues of IRF-7 DN (237–514) were not the targets for virus-induced phosphorylation or that phosphorylation of S483 and S484 is a prerequisite for further phosphorylation of IRF-7. It was shown that in IRF-3, S385 and S386 regulated the phosphorylation of a downstream serine/threonine cluster (Lin et al., 1999). Maniatis and coworkers have shown that IRF-7A and IRF-7B are constitutively phosphorylated in HEC-1B cells and only a marginal increase in phosphorylation was detected upon virus infection (Whateleff et al., 1998). In contrast, we observed a significant increase of phosphorylation of IRF-7 DN (237–514) in infected 2FTGH cells. Most likely, this discrepancy reflects different constitutive levels of activated kinase in these two cell types.

Focusing on the characterization of IRF-7 functional domains required for the expression of endogenous IFNA genes in human 2FTGH cells, we have found that only full-length IRF-7 can activate IFNA gene expression in the infected cells. Overexpression of the IRF-3/p65 fusion protein or IRF-7 C-terminal truncation mutants (1–237 and 1–349) which activated IFNA4 promoter in a transient reporter assay (Au et al., 1998; Schafer et al., 1998) did not support transcriptional activation of the endogenous IFNA genes.

Several IRF family members were shown to interact with each other through the IAD and as a result, their DNA-binding activity can be modulated (Sharf et al., 1997). By using the GST pull-down assay, we have shown that IRF-3 and IRF-7 can directly interact with each other. The IRF-7 (237–418) which does not bind IRF-3 in vitro failed to modulate the virus-mediated induction of IFNA genes. The observation of interaction between the N-terminal half of IRF-7 (1–237) and IRF-3 was unexpected. Interestingly, this region contains a potential PEST domain (RGGPPPEAETAER) which has a score (+8.07) higher than that of PU.1 (PESTfind). The interaction between PEST domain of PU.1 and IAD of IRF-4 and IRF-8 was previously demonstrated (Meraro et al., 1999). This PEST domain is unique to IRF-7 and is absent in all the other IRFs. Furthermore, it has been suggested recently that it may contribute a low stability to IRF-7 protein (Sato
et al., 2000). The interaction between the C-terminal part of IRF-7 and IRF-3 occurs through IAD-like domains and it is greatly enhanced by virus-induced phosphorylation. It was shown that mutations of L331P and G377D in IAD of IRF-8 prevented the complex formation between IRF-8 and IRF-1 or IRF-2, as well as its binding to PRDI (Meraro et al., 1999). Interestingly, these two amino acids are conserved both in the interacting domains of IRF-7 (418–473) and IRF-3 (306–427) and are deleted in IRF-7 (237–418), an IRF-7 deletion mutant that can neither bind IRF-3 nor inhibit IRF-7-mediated induction of IFNA genes.

Altogether these data indicate IRF-3 and IRF-7 heterodimers are effective transcription activators of IFNA genes in infected cells and that IRF-7 DN (237–514) targets both IRF-3 and IRF-7. The affinity of IRF-7 DN (237–514) for IRF-3 is significantly enhanced by virus-induced phosphorylation of IRF-7DN on S483 and S484. While both IRF-3 and IRF-7 were shown to bind DNA as homodimers, our data indicate that binding of IRF-7DN to IRF-7 or IRF-3 significantly decreases the DNA binding capacity of IRF-3 and IRF-7. This may be, however, only one of the mechanisms by which IRF-DN inhibits the induction of Type I IFN genes in infected cells, since the interaction of IRF-3 and IRF-7 with transcription coactivators CBP/p300 is likely to be altered as well in the presence of IRF-7 DN (237–514). This possibility has been examined.

**MATERIALS AND METHODS**

**Cells, virus, and transfection.** 2FTGH cells, L-929 and 293 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. L-929 cells constitutively expressing IRF-7 DN (237–514) were established by transfecting L-929 cells with IRF-7 DN (237–514) expressing plasmid, followed by over 2 weeks of G418 selection. The resistant clones were pooled and used in the study. All the transfections were done using Superfect transfection reagent (Qiagen Inc.). Routinely, 1 μg of RNA sample was reverse-transcribed into cDNA using oligo-dT (12–18) as primer in 30 μl final volume and 2 μl of the RT reaction mixture was used for PCR. Primer sets that amplify murine (Mu)β-actin (36), MuIFNα and MuIFNβ (35), human (Hu)β-actin, HuIFNA, and HuIRF-7 (30, 34) were previously described. The following primer sets are used for amplifying HuIFNB (5’ primer, TTGTGCTTCTCACATCAGC and 3’ primer, CTGTAAGTCGGTTATCAG) and HuIRF-3 (5’ primer, CTGAAGCGGCTGTTGGTG and 3’ primer ACCATAGGCAGCG). The amount of cDNA used and the number of cycles of amplification were determined to be within the linear range of amplification.

**Metabolic labeling of cellular proteins with 32Pi.** 293 cells were transfected with 5 μg of plasmid encoding either IRF-7 DN (237–514) or its corresponding phosphorylation mutant (IRF-7 DN ph-). Sixteen hours posttransfection, medium was changed to a phosphate-free DMEM supplemented with 2% dialyzed serum for 2 h. Cells were then infected with Sendai virus and labeled with 0.5 m Ci/ml of 32Pi (Amersham Inc.) for 4 h. The whole-cell extract (300 μg) was used for immunoprecipitation with anti-Flag antibody and precipitated proteins were resolved on 10% SDS–PAGE.
**DNA-pull-down assay.** Double-stranded (ds) oligomers corresponding to the IFNA1 VRE region (−110 to −53 bp) were synthesized with biotin at the 5′ end of the antisense strand; the ds oligomers were incubated with streptavidin magnetic beads (Dynal Inc.) for 1 h in TEN buffer (20 mM Tris pH 8, 1 mM EDTA, 0.1 M NaCl). After incubation, the unbound DNA was removed by extensive washing with TEN buffer. Nuclear and cytoplasmic extracts pooled and dialyzed against the binding buffer containing 10% glycerol, 12 mM Hepes, pH 7.9, 5 mM MgCl₂, 60 mM KCl, 0.1 mM DTT, and 0.1 mM PMSF were then incubated with the DNA bound magnetic beads for 4 h at 4°C. After gentle washing, the bound proteins were resolved by SDS-PAGE and proteins were identified by the Western blot with the indicated antibody.

**IFN assay.** The cytopathic assay was used to determine the level of Type I IFN in the medium as described previously (Cheung et al., 1991). VSV (moi. 5) was used as challenging virus and human foreskin fibroblasts as indicator cells. The IFN titer in the medium was calculated based on the antiviral activity relative to that conferred by the IFN standard.

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