A Type I Interferon Signaling Factor, ISF21, Encoded on Chromosome 21 Is Distinct from Receptor Components and Their Down-regulation and Is Necessary for Transcriptional Activation of Interferon-regulated Genes

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The type I interferons (IFNs) are a family of cytokines, comprising at least 17 subtypes, which exert pleiotropic actions by interaction with a multi-component cell surface receptor and at least one well characterized signal transduction pathway involving JAK/STAT (Janus kinase/signal transducer and activator of transcription) proteins. In a previous report, we showed that a signaling factor, encoded by a gene located on the distal portion of chromosome 21, distinct from the IFNAR-1 receptor, was necessary for 2'-5'-oligoadenylate synthetase activity and antiviral responses, but not for high affinity ligand binding. In the present studies using hybrid Chinese hamster ovary cell lines containing portions of human chromosome 21, we show that the type I IFN signaling molecule, designated herein as ISF21, is distinct from the second receptor component, IFNAR-2, which is expressed in signaling and non-signaling cell lines. The location of the gene encoding ISF21 is narrowed to a region between the 10q21 and the r21 breakpoints, importantly eliminating the Mx gene located at 21q22.3 (the product of which is involved in IFN-induced antiviral responses) as a candidate for the signaling factor. To characterize the action of this factor in the type I IFN signaling pathway, we show that it acts independently of receptor down-regulation following ligand binding, both of which occur equally in the presence or absence of the factor. In addition, we demonstrate that ISF21 is necessary for transcriptional activation of 2'-5'-oligoadenylate synthetase, 6-16, and guanylate-binding protein gene promoter reporter constructs, which are mediated by several signaling pathways. ISF21 represents a novel factor as the localization to chromosome 21, and the data presented in this study exclude any of the known type I IFN signal-transducing molecules.

The type I interferons (IFNs)\(^\dagger\) are a family of species-specific, multifunctional cytokines, which in humans include 15 subtypes of IFNα with 75–98% amino acid identity, IFNω with 70% identity to consensus IFNα, and the least related IFNβ with 35% identity to IFNα (1). Despite quantitative differences in biological specific activities among type I IFN subtypes (2) and differences in antigenicity (3), they all induce similar biological functions in human cells (4) and compete for binding to cell surface receptors (5).

The first cloned component of the human type I IFN receptor (designated as IFNAR-1), when expressed in mouse BTG9A cells, appeared to selectively mediate responses to a restricted range of type I IFNs: only IFNα but not IFNω or IFNβ (6). The inability of IFNAR-1-transfected cells to respond to all IFNs may have been due to the absence of other human receptor components and a difference in the ability of these subtypes to interact with (other) murine receptor components. Indeed, the definition of the role of IFNAR-1 in ligand binding has been complicated by the differences in results obtained when the receptor was expressed in different types of host cells.

Recently, a second IFN receptor component (encoded by a gene designated as IFNAR-2) was identified and shown to exist as a soluble form (IFNAR-2a) and a transmembrane form with a short cytoplasmic domain (IFNAR-2b) (7). This component was shown to bind type I IFNs α, β, and ω, and β by cross-linking experiments, and when co-expressed with IFNAR-1 in murine cells bound \(^{125}\)I-IFNα with an affinity of \(-300\) pM. However, the function of IFNAR-2b in signal transduction was unclear (7).

Recently, it has been shown that the IFNAR-2 gene codes a third form with a longer cytoplasmic domain, designated as IFNAR-2c, which mediates signaling when co-expressed with IFNAR-1 in murine L929 cells (8, 9).

IFNAR-1 has been localized to human chromosome 21 in the region 21q22.1 (5, 6, 10). We recently showed using a panel of CHO-human chromosome 21 hybrid cells that there is a gene(s) encoded in the region 21q22.2–3, and therefore distinct from IFNAR-1, that is necessary for type I IFN signal transduction (11). Cells containing human chromosome 21 proximal to the 8;21 breakpoint (21q+) expressed the mRNA for IFNAR-1 and bound IFNs αB, α2, and β with an affinity of approximately 200 pM, indicating that the region 21q22.1 contained factors, in addition to IFNAR-1, required for ligand binding. However, unlike cells that contained the entire chromosome 21, the 21q+ cell line did not signal as measured by induction of 2'-5'-oligoadenylate synthetase enzyme activity and antiviral responses.

In the present study, we show that the type I IFN signaling factor encoded on human chromosome 21, now designated as
ISF21, is distinct from IFNAR-2 as well as IFNAR-1 receptor components, both of which are expressed in the hybrid cell lines, including those that do not signal. The Mx gene was a candidate for the signaling factor by virtue of it exhibiting properties of a signaling molecule and its ability to induce an antiviral state and its location on human chromosome 21q22.3. In the present study, the location of the gene encoding ISF21 is narrowed to a 400-kb region between the 10;21 and r21 breakpoints which is proximal to the Mx locus. Despite the lack of signaling, hybrid cells containing the IFNAR-1 and -2 genes but not containing ISF21 are shown to bind a range of IFN subtypes, which compete with each other and undergo ligand-dependent down-regulation of IFN binding sites. Thus the signaling factor ISF21 acts independently of ligand-receptor interaction, processing, and receptor down-regulation. Importantly, we also demonstrate, using a range of IFN-sensitive reporter constructs, that the signaling factor ISF21 is necessary for signaling pathways prior to activation of IFN-responsive genes.

MATERIALS AND METHODS

Cell Lines and Interferons—The parental CHO-K1 cell line was obtained from the American Type Culture Collection. The following CHO-human chromosome 21 containing hybrid cell lines were obtained from D. Patterson (Eleanor Roosevelt Institute, Denver, CO): 21q+, MRC 2G, 10;21 (5942C-5a), 6918–8a1, R2–10W, 21;22 (RAJ-5), 643C-13 (7; 21), and 7253Sx6. The human chromosomal complement of the hybrids has been described elsewhere (12) and is summarized in Fig. 2. The CHO-K1 cell line was stably transfected with the human IFNAR-1 cDNA contained in an expression vector controlled by the sheep metallothionein promoter (pTV2, 13), by electroporation at 960 microfarads and 270 V. Several independent clones were expanded, and expression was confirmed by RT-PCR (data not shown). All cell lines were grown in RPMI 1640 medium supplemented with 5% dialyzed fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, except CHO-K1 and 21q+ cultures, which were also supplemented with 2.3 mg/ml proline. HeLa cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin. The IFNs used in this study were huIFNα2a (Hoffman La Roche, Basel, Switzerland), huIFNβ (Ciba Geigy, Basel, Switzerland), huIFNβ3a (Berlex Laboratories, Alameda, CA), huIFNα1c (Amgen, Thousand Oaks, CA), and Wellferon (human lymphoblastoid IFN, Wellcome Laboratories, UK). IFNα4 was transmitted using SP6 polymerase and translated using rabbit reticulocyte lysate as described previously (14); control experiments using rabbit reticulocyte lysate only had no effects on gene induction in the hybrid cell lines (data not shown). For receptor binding studies, the IFNs were iodinated using modified chloramine-T procedures to a specific activity of ~100 μCi/μg and the integrity of the IFN was monitored as described previously (11, 15).

Receptor Binding and Down-regulation—Receptor binding assays were performed essentially as described previously (11). Scatchard analysis of binding curves was performed using the LIGAND program and was found to be statistically significant (p < 0.05) only when resolved by a “one-site” fit. Competitive binding experiments were performed using 400,000 cpm of 125I-IFNαB and 1, 5, 10, 30, 100, and 300-fold excess IFNαB, α2 and β, essentially as described previously (16).

The down-regulation of ligand binding sites on the cell surface was determined using 125I-IFNα1c essentially as described previously (17). The number of receptors per cell was determined by a conventional ligand binding assay and Scatchard analysis before and after incubation with 20,000 IU of this IFN/ml for 18 h. A similar study was undertaken using an 125I-4B1 monoclonal anti-IFNα1 antibody (17) to measure the number of IFNAR-1 chains.

Isolation of Human IFNAR-2 cDNA—Primers were generated using the published IFNAR-2 cDNA sequence (7), spanning the regions 219–240 bp and 1203–1222 bp, which encompass the ATG and TGA codons, respectively. Reverse transcription was carried out using 5 μg of total RNA from human Daudi cells using avian myeloblastosis virus reverse transcriptase (Promega) at 42 °C and the antisense primer. PCR was subsequently performed on the cDNA under the following conditions: 93 °C for 60 s, 56 °C for 60 s, and 72 °C for 90 s for 35 cycles. The PCR product was electrophoresed on a 1% agarose gel, and a band of the expected size of 1003 bp was observed. The PCR product was cloned into pGEM-T (Promega) and sequenced, using an automated DNA sequencer (Applied Biosystems).

Northern Blots—Cells were grown to mid-log phase, harvested, and poly(A)+ mRNA extracted as described previously (18). Approximately 9 μg of RNA in 50% formamide was electrophoresed on 1% agarose formaldehyde gels, transferred to Hybond C membranes (Amersham) in 20 × SSC overnight. Filters were then baked at 80 °C for 2 h and prehydrated at 42 °C for 2–3 h. The filters were hybridized with a 32P-labeled IFNAR-2 cDNA probe as described previously (11), stripped, and reprobed overnight at 42 °C with a 32P-labeled 1.1-kb fragment of the glyceraldehyde-3-phosphate dehydrogenase cDNA as a control for RNA loading. After hybridization and washing in 0.1 × SSPE, 0.1% SDS at 65 °C, signals were visualized by autoradiography on Kodak BioMax film.

Analysis of IFN-stimulated Gene Promoter Activity in Hybrid Cell Lines—To construct a plasmid containing the 2′-5′-OAS promoter-CAT reporter (25A-CAT), a human 2′-5′-oligoadenylate synthetase gene promoter fragment corresponding to residues 525–1435 in the published sequence (19) was generated by PCR using oligonucleotides 5′-GAAC-TCCTGCTGACATTCACGCG-3′ and 5′-GGAGAACACUFPCFTGCGAAAC-3′ and cloned into the pCR II vector (Invitrogen Corp.). A SpeI restriction site was then created 18 bp 5′ of the ATG by PCR. A XbaI-SpeI fragment encompassing ~834 to ~29 of the 2′-5′-OAS promoter was then cloned into the XbaI site of pCATBasic (Promega). Reporter constructs containing a human 6–16 promoter fragment (fragment no. 3 in Ref. 20) were constructed by digesting the promoter from P. Rathjen, Department of Biochemistry, University of Adelaide) with HindIII and then end-filling with Klenow (Promega). After digestion with BglII, the fragment was ligated into the vector pGL3-Basic (Promega) that had been digested with SmaI and BglII, to give the p30X-Luc construct. The vector had also been modified to contain a neomycin resistance gene, derived from pMC1Neo, that had been inserted into the unique SfiI site of pGL3-Basic. The GFP reporter-promoter-luciferase construct (GBP-LUC) was a gift from B. R. G. Williams (Cleveland Clinic Foundation, Cleveland, OH).

For analysis of reporter activity, cells were diluted to 1 × 105 cells/ml in electroporation buffer (20 mM Hepes, 137 mM NaCl, 5 mM KCl, 0.7 mM Na2HPO4, 6 mM glucose, 0.1 mM 2-mercaptoethanol, pH 7.0) and 0.5 μl of annealed and ligated with 5 μg of psi-β-galactosidase Control vector (Promega) before electroporation at 960 microfarads and 270 V in a Gene Pulser (Bio-Rad). Cells were plated in 10-cm dishes and allowed to recover overnight before incubation with 100 IU/ml various type I IFNs (as indicated) for 16 h. Cells were then harvested, sonicated in 250 mM Tris, pH 8, and 20 μl of clarified lysate incubated as described previously for CAT activity (21). For luciferase assays, cells were lysed directly in reporter lysis buffer (Promega) and luciferase light units measured using a Promega Luciferase kit and a Berthold luminometer.

As a control for transfection efficiency, β-galactosidase enzyme activity was determined by incubation of 30 μl of clarified lysate, prior to heat inactivation, with 2 × β-galactosidase buffer (200 mM NaPO4, pH 7.3, 2 mM MgCl2, 100 mM β-mercaptoethanol, 1.33 mg/ml o-nitrophenyl-β-D-galactopyranoside) in a total volume of 100 μl, at 37 °C for 30 min, and the absorbance at 415 nm read using a microplate reader (Bio-Rad). The CAT activity was determined as the percent of substrate converted to product, then expressed relative to the β-galactosidase activity for the same sample. Results were shown as -fold induction by IFN relative to untreated controls. Luciferase light units were determined relative to β-galactosidase enzyme activity and expressed as -fold induction by interferon relative to untreated controls.

Induction of 2′-5′-Oligoadenylate Synthetase Activity—Cells were incubated with 0 or 1000 IU/ml IFN for 48 h before being harvested and lysed. Enzyme activity was determined by the incorporation of [γ-32P]ATP into alkaline phosphatase-resistant 2′-5′-oligoadenylate-resistant “cores,” as described previously (22).

RESULTS

Expression of IFN Receptors in Signaling and Non-signaling Hybrid Cell Lines—To determine whether the lack of signaling observed previously in the 21q+ cell was due to the absence of IFNAR-2, we performed Northern blot analysis (Fig. 1). A full-length cDNA probe for IFNAR-2a (7) was generated by RT-PCR using human Daudi cell total RNA. It is noteworthy that the sequence of the IFNAR-2a cDNA was identical to the published sequence (7) except for 3 nucleotides. At nucleotide 700, a change from C to T would result in an amino acid change from Pro to Ser at amino acid residue 212; at nucleotide 859, an
A to G change would result in a change from Thr to Ala at residue 265, while a change from T to C at nucleotide 501 would be silent. Using this cDNA as a probe, IFNAR-2 mRNA transcripts were detected in all of the human chromosome 21 hybrid cells, which had previously been reported to also express IFNAR-1 (11), but not in the parental CHO cells. As an example, Fig. 1 shows a Northern blot analysis of poly(A)+ mRNA from one signaling (72532x6) and one non-signaling (21q+) cell line and the parental CHO-K1 cells. Two transcripts of approximately 4.5 and 1.5 kb were observed, consistent with published data (7). This result indicates that the IFNAR-2 gene is encoded on human chromosome 21, in the region 21p-q22.1, as is IFNAR-1, and both are expressed even in the hybrid cell lines which do not signal (see below, and Ref. 11).

**Location of the Gene Encoding the Signaling Factor ISF21 to a 400-kb Region on Human Chromosome 21q22.2—**Previous studies had described the location of a gene encoding a type I IFN signaling factor to be on the distal third of chromosome 21, distal to the 8;21 breakpoint. This region of human chromosome 21 contained the Mx genes, which possess GTPase activity and contain Zn finger motifs characteristic of signaling molecules, and are necessary for some antiviral responses to type I IFNs. Therefore it was important to determine whether ISF21 could be distinguished from Mx and at the same time, to narrow down the region containing this gene to facilitate further cloning studies. We therefore examined an extended panel of CHO-human chromosome 21 hybrid cell lines which contained smaller chromosomal deletions (Fig. 2). Induction of 2'-5'-OAS enzyme activity was observed after IFN treatment in cell lines containing human chromosome 21 fragments which extended further than the r21 breakpoint, namely R2–10W, RAJ 5, 643C-13, and 72532x6. However, no induction of 2'-5'-OAS was observed in hybrid cell lines which contained only human chromosome 21 sequences proximal to the 10;21 breakpoint, namely 6918–8a1, MRC 2G, and 21q+. This was despite the observation that these non-signaling cell lines expressed the genes encoding both known type I IFN receptor components. Therefore, the gene encoding the signaling factor ISF21 is located between the 10;21 and r21 breakpoints, and is thus distinct from the IFN receptor locus which lies in the region between the 6918 and 8;21 breakpoints (9). Furthermore, the signaling factor designated ISF21 is not the Mx gene, which would be absent from the R2–10W cell line, whereas this cell line does transduce signals.

Recently an arginine methyltransferase, termed IRIB4, has been shown to associate with the type I IFN receptor and was implicated in IFN signaling (23); interestingly, a related arginine methyltransferase, hHMT 1, was localized to human chromosome 21 (GenBank™ accession no. X99209). We generated a probe for the latter gene for Southern blot analysis of the panel of hybrid cell lines. This gene was detected in the 643C-13 cell line, but not in 21q+, MRC-2G, 6918–8a1, or RAJ 5 (data not shown) and therefore did not fit the pattern of expression of ISF21.

**ISF21 Is Required in the IFN Signal Transduction Pathway prior to Transactivation of IFN-responsive Genes—**Previous studies had indicated that the type I IFN signaling factor was...
required for induction of 2'-5'-OAS enzyme activity (11), but it was not known at which stage in the IFN-dependent increase of this enzyme this factor acted. To better define the nature of this factor, we set out to determine at what stage of IFN signaling the factor acted and whether it was involved in the induction of other IFN-responsive genes. First, a 910-bp fragment from the 2'-5'-OAS promoter region, which contains all the elements necessary for the induction of transcription of this gene, was ligated upstream of a CAT reporter gene (25A-CAT). The CHO-K1, 21q+, and 72532x6 cell lines were transiently transfected with 25A-CAT and cotransfected with a β-galactosidase construct as a control for transfection efficiency. After treatment with various human type I IFNs, the parental CHO and 21q+ cell lines showed no significant induction of CAT activity, apart from a weak induction with huIFNβ due to a low level of reactivity with hamster cells (Fig. 4A). All type I IFNs tested, namely α2, α4, αβ, Wellferon, and β, induced CAT activity in the 72532x6 cell line but not in the 21q+ cell line (Fig. 4A), again emphasizing that ISF21 is necessary for signaling in response to a broad range of type I IFNs. Treatment of the same three cell lines with murine IFNα4 resulted in induction of the reporter to a similar extent in all three cell lines consistent with this IFN acting through the hamster receptors. This result importantly demonstrates that all of the components necessary for transcriptional activation of the 2'-5'-OAS-reporter are present in these cells; but they cannot be activated through the human type I IFN receptor in the absence of ISF21.

To determine if ISF21 was also necessary for the transcriptional activation of other IFN-responsive genes, two other ISGs were analyzed using this system. The 6–16 gene promoter was shown to be responsive to huIFNαB in the 72532x6 cells, which contain ISF21, but not in 21q+, which lack this factor (Fig. 4B). Interestingly, the level of induction of 2'-5'-OAS and 6–16 reporter constructs was similar, namely 4–5-fold, and both genes are known to be inducible via ISGF3 binding to ISRE elements. The third promoter construct used in this study, the GBP-LUC, was chosen because GBP is reportedly induced independently of ISGF3 binding to the ISRE, but instead through the IRF-1 and NF-κB transcription factors (25). Although only a low level of induction was observed in the 72532x6 cell line by IFNαB, it was similar to that detected in the human HeLa cell line (Fig. 4C). Importantly, no induction was observed in the 21q+ nor the CHO K1 cell lines (Fig. 4C), indicating that the
The data shown are the mean of triplicate determinations in a representative one of three independent experiments. The coefficient of variation was less than 15% of the mean. mAb, monoclonal antibody.

| Cell Line | Binding of 125I-IFNa | Binding of 125I-IFNAR-1 mAb |
|-----------|---------------------|-----------------------------|
| 21q+      | 1160                | 860                         |
| 72532x6   | 1275                | 900                         |

**DISCUSSION**

The data presented herein establish several important points about the type I IFN signaling molecule, designated as ISF21. 1) It is distinct from the receptor component IFNAR-2 as well as IFNAR-1. 2) It acts independently of down-regulation of the functional receptor subsequent to ligand binding. 3) It is localized to a 400-kb region on human chromosome 21 between the 10;21 and r21 breakpoints and thus distinguished from the Mx gene, which encodes an IFN inducible antiviral molecule, and from an arginine methyltransferase gene related to a proposed IFN signaling molecule. 4) It is essential for the induction of the interferon-inducible genes 2'-5'-oligoadenylate synthetase, 6–16, and guanylate-binding protein and therefore probably involved early in signal transduction for activation of several pathways for induction of IFN-responsive genes.

Human IFNAR-2 cDNA was generated by RT-PCR using RNA from Daudi cells and used to demonstrate that hybrid cell lines containing portions of chromosome 21 contained the IFNAR-2 gene and expressed both mRNA transcripts observed for this gene. We had previously shown by direct binding studies that these cells bind human type I IFN ligands with an affinity of approximately 200 pM and that the affinity and number of binding sites are not affected by the presence or absence of the signaling factor. Interestingly, hamster cells containing a yeast artificial chromosome expressing both IFNAR-1 and IFNAR-2 (26), or murine cells containing cDNA for human IFNAR-1 and -2 (27) also bind type I IFNs with affinities of 200–300 pM. In CHO cells, we found that IFNAR-1
alone was insufficient to enable any detectable binding of human type I IFNs. Therefore the products of these two genes are necessary and may account for all the receptor components necessary for binding to the type I IFN ligands. The conflicting data previously reported on the necessity of huIFNAR-1 for the binding of type I IFN ligands (6, 8, 9, 28) probably reflect the requirement for more than one component for binding and the variable ability of different type I IFNs to interact with other endogenous receptor components in non-human cells. The fact that IFNs α2, αβ, and β (the least homologous type I IFN) compete for binding to the 21q+ cells, albeit with different affinities, indicates that they share at least one, probably both of these chromosome 21-encoded binding components, and that competition between type I IFNs is not influenced by the signaling factor ISF21.

Although hybrid cells containing IFNAR-1 and -2 genes bind type I IFNs, they do not signal unless the distal portion of chromosome 21 is present as evidenced by our studies on three independent non-signaling cell lines. There have been reports of similar situations in human cell lines that bind IFN but are insensitive to the biological actions of IFNs; these cell lines did not efficiently down-regulate the receptor-ligand complex (29). It was therefore possible that the signaling factor described herein might be involved in down-regulation of receptors. Our data clearly show that this was not the case, since a signaling (72532x6) and non-signaling (21q+) hybrid cell line both down-regulated the type I IFN receptor complex to similar levels, using two different methods to measure this phenomenon. Thus ISF21 is not required for the down-regulation process, and acts either at a step in signal transduction that occurs after the down-regulation of the ligand-receptor complex or independently of it.

Our previous data showed that ISF21 is necessary for induction of 2'-5'-OAS enzyme activity by IFNs α2, αβ, and β (11). However, it was not clear whether this signaling factor was necessary for transcriptional activation of this IFN-responsive gene or if it was involved in post-transcriptional regulation of enzyme activity. The data presented herein using the 2'-5' OAS promoter-CAT reporter construct demonstrated that the ISF21 was necessary for transcriptional activation of this ISG in response to many type I IFNs, indicating its importance in signaling by probably all type I IFNs. Our results may seem to be in apparent contradiction to other reports using murine (8) or human (9) cells, wherein a combination of human IFNAR-1 and IFNAR-2 products is sufficient for binding and signaling in response to IFN (8, 9). An explanation for this discrepancy could be that the hamster ISF21 does not interact with human IFN receptors, whereas the murine ISF21 does. This explanation is consistent with observations on human IFN binding to human IFNAR-1, which occurs when expressed in murine cells but not in CHO cells (see below and Ref. 26), suggesting a species specificity for facilitating ligand binding in mouse but not hamster cells. It is important to note that signal transduction through the endogenous hamster components could be detected since murine IFNα induced the 2'-5'-OAS-CAT reporter (and 6–16 as well) in CHO-K1 as well as 21q+ and 72532x6 hybrid cell lines. This demonstrates that all of the “downstream” components required for transcriptional activation are present in the hybrid cell lines, but that human ISF21 is necessary, in a species-specific manner, for transactivation of IFN-responsive genes.

To examine the scope of ISF21 action in IFN signaling, we examined the responsiveness of the two other IFN-responsive genes, 6–16 and GBP. The 6–16 gene promoter, like the 2'-5'OAS contains multiple IFN-responsive elements, ISRE, interferon response element, and γ activated sequence (25), but are mainly inducible by type I IFNs via ISGF3 binding to the ISRE (25). The GBP promoter, although more weakly inducible by IFNs than by IFNγ, is regulated by IRF-1 and NF-κB rather than ISGF3 (25). Importantly, all three promoter constructs were dependent on ISF21 by virtue of their induction in 72532x6 cells but not in 21q+ or CHO K1 cells. These data indicate that ISF21 is necessary for several signaling pathways activated by type I IFNs. This conclusion is supported by the necessity of ISF21 for an antiviral response in these cells, which could be achieved via multiple signaling pathways or ISGs.

MX genes, located on the distal portion of human chromosome 21 were candidates for ISF21, since these are known to be induced by type I IFN, are involved in signal transduction. In these studies we have narrowed the chromosomal localization of ISF21 to the region on chromosome 21 between the 10:21 and the r21 breakpoint, indicating that the Mx gene is not ISF21. There was also circumstantial evidence for an arginine methytransferase involvement in IFN signaling (23), but this was also excluded on the basis of gene mapping in signaling and non-signaling cell lines. Furthermore, since none of the well characterized IFN signaling molecules such as JAKs (Janus kinase) and STATs (signal transducer and activator of transcription) are encoded by genes on chromosome 21, ISF21 is likely to be a novel IFN signaling factor. Its cloning will be facilitated by the narrowing of the location of its gene reported herein.

Thus, as shown in Fig. 2, there is a cluster of genes associated with IFN response found on human chromosome 21, which include IFNAR-1, IFNAR-2, CRF2-4, IFNGR-2, ISF21, MX-1 and MX-2. The factors encoded by these genes are essential for biological responses to IFNs including the regulation of cell proliferation and differentiation, and immune responses. It is interesting to note that in Down syndrome, where there is trisomy of chromosome 21, there is retarded growth and perturbations of the immune system, which may be in part due to altered regulation of the various IFN response genes located on this chromosome.

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Type I IFN Signaling Factor, ISF21

21051

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