The Structure of the Gene for the Second Chain of the Human Interferon-γ Receptor*

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The gene for the second chain of the human interferon-γ receptor was analyzed from cosmid DNA clones. The gene spans over 33 kilobases of DNA and contains seven exons. The signal peptide is encoded by exons 1 and 2, the extracellular domain by exons 2, 3, 4, 5, and by part of 6. Exon 6 also encodes the whole transmembrane domain and part of the intracellular domain. Exon 7 encodes the remainder of the intracellular domain and contains the 3'-untranslated region. The sequences at the exon/intron boundaries are well conserved with respect to canonical acceptor/donor sites (AG/GT). The 5'-flanking region was sequenced and analyzed for transcription factor binding sites. No TATA or CAAT boxes in the promoter region were identified. Consistent with the lack of a TATA box, analysis of the mRNAs by primer extension showed multiple transcription start sites. Promoter activity of the 5'-flanking region was investigated with a luciferase reporter gene and the cytomegalovirus minimal promoter. Segments of the 5' region with promoter activity were identified.

Interferon-γ (IFN-γ) exerts its pleiotropic biological function by binding to its cell surface receptor. The IFN-γ binding chain (IFN-γR1) of the human (Aguet et al., 1988) and mouse (Kumar et al., 1989; Hemmi et al., 1989; Cofano et al., 1990; Munro and Maniatis, 1989; Gray et al., 1989) receptor were cloned. It was shown that IFN-γR1 binds IFN-γ, but failed to generate a biological response (Rashidbaig et al., 1986). Jung et al. (1987, 1988) demonstrated that an additional component located on human chromosome 21 was required to induce a biological function in response to IFN-γ. In mouse cells, the comparable component was located on mouse chromosome 16 (Hibino et al., 1991). This accessory factor (AF-1) or second chain of the IFN-γ receptor complex, IFN-γR2, was cloned (Soh et al., 1994; Hemmi et al., 1994). The IFN-γR2 chain could support the biological responses of IFN-γ in the presence of the IFN-γR1 chain. However, it was suggested that additional factors might still be required because of the lack of ability of the IFN-γR2 chain to restore all biological functions (i.e. in particular, antiviral activity) fully (Soh et al., 1994; Cook et al., 1994).

The IFN-γR receptor complex requires Janus kinase-1 (Jak1) and Jak2 for signal transduction (Müller et al., 1993). Kotenko et al. (1995) demonstrated that Jak1 and Jak2 are associated with the intracellular domain of the IFN-γR1 and IFN-γR2 chains, respectively. In addition, Jak1, Jak2, and IFN-γR1 are phosphorylated in response to IFN-γ in cells expressing both IFN-γR1 and IFN-γR2. Upon phosphorylation of the kinases, Jak1 and Jak2 are activated. The expression of the IFN-γR2 chain was absent in type 1 T helper cells (T₃₂₁) and appears to be down-regulated by IFN-γ (Pernis et al., 1995; Bach et al., 1995; Skrenta et al., 1996). Thus, defining the structure of the IFNγR2 gene and its promoter, which is the subject of this report, should help to understand how this chain is regulated in these cells and in others.

EXPERIMENTAL PROCEDURES

Reagents, Restriction Endonucleases, and Other Enzymes—All restriction endonucleases were from Boehringer Mannheim Biochemical Corp. The [γ-³²P]ATP (α-³²P)dATP were from DuPont NEN. All other chemical reagents were analytical grade and purchased from United States Biochemical Corp.

Plasmid Vectors and Preparation of Plasmid DNA—Plasmid pBluescriptII KS⁺ (pBS) (Stratagene) was used for subcloning of genomic DNA for sequencing. For the assay of promoter activity of the human IFNγR2 gene, various sizes of fragments were inserted 5’ upstream of the luciferase gene of the plasmid pUHC-13-3 (Gossen and Bujard, 1992) without the tetracycline-operator sequence. Because the plasmid pUHC-13-3 contained 335 nucleotides of heptamerized tetracycline operators followed by the cytomegalovirus (CMV) minimal promoter containing a TATA box upstream of the luciferase gene, it was necessary to remove the tetracycline operators to use the vector as a luciferase reporter plasmid. The tetracycline operators were removed by digesting with restriction endonucleases XhoI and StuI. The plasmid fragment was blunt-ended, recircularized by ligation, and the resultant plasmid was designated pSR2. As a positive control for the luciferase activity, the complete CMV promoter was isolated from plasmid pUHD-15-1 (Gossen and Bujard, 1992) by digestion with restriction endonucleases XhoI and EcoRI. The fragment containing the CMV promoter was inserted into plasmid pUHC-13-3 that was digested with restriction endonucleases XhoI and StuI by ligating the matching XhoI ends, then filling in the remaining EcoRI and StuI sites, and then recircularizing. The resultant plasmid was designated as pSR2. Plasmid DNA was prepared by the alkaline lysis method (Sambrook et al., 1989).

DNA Sequencing and Analysis of Sequences—DNA sequencing was performed by the dideoxynucleotide chain termination method with Sequenase Version 2.0 DNA sequencing reagents (U.S. Biochemical Corp.) following the manufacturer's protocol. Sequences were analyzed with the Wisconsin GCG software package (Devereux et al., 1984).

Primer Extension—Primer extension was carried out as described previously (Sambrook et al., 1989). The sequence of oligonucleotide 6799 (5’-GCCGCCCACACCGGCATGCGC-3’) was from nucleotide 93.
Structure of the Gene for Second Chain of Hu-IFN-\(\gamma\) Receptor

![Gene structure of the second chain of the human interferon-\(\gamma\) receptor](image)

At the top, two cosmid clones which were used for sequence analysis are shown. The restriction endonuclease EcoRI sites which were used to subclone the cosmid fragments are shown on the gene. The exons and introns are numbered, and the sizes of introns are shown. pJS3, a shorter cDNA clone, is 1668 bp long and pSK1 is 2553 bp long (Soh et al., 1994). 5'-UT, 5'-untranslated region; SP, signal peptide; EC, extracellular domain; TM, transmembrane domain; IC, intracellular domain.

to nucleotide -73 upstream of the ATG translation start site of the Hu-IFN-\(\gamma\)-R2 cDNA in the 3' to 5' direction (Soh et al., 1994; see also Fig. 1 and Fig. 2). oligonucleotide 7513 (5'-GTCGAGGGTGGAGGG-GATT-3') was from nucleotide -157 to nucleotide -140 upstream of the ATG start site of clone pSK1 (Soh et al., 1994); oligonucleotide 7531 (5'-AGCTCCCGGAAGCTTCTG-3') was from nucleotide -327 to nucleotide -310 upstream; oligonucleotide 7583 (5'-AAGTACATATATAGGTTT-3') was from nucleotide -546 to nucleotide -529 upstream; and oligonucleotide 7757 (5'-GAGAGACAGAAACTTCCA-3') was from nucleotide -769 to nucleotide -752 upstream. Each oligonucleotide was end-labeled with [\(\gamma\)-\(\alpha\)P]ATP and T4 polynucleotide kinase. Then, the end-labeled primer (10^6 cpm) was coprecipitated with 20 \(\mu\)g of total RNA (prepared as described by Kumar et al. 1989) and redissolved in formamide-containing hybridization buffer. This mixture was heated at 85 \(^\circ\)C for 10 min and allowed to hybridize overnight at 30 \(^\circ\)C. Following hybridization, the mixture was precipitated with ethanol and dissolved in 20 \(\mu\)l of reverse transcription buffer containing 1 mM each of dNTP, 1 mM dithiothreitol, 270 mM (MgCO\(_3\))\(\cdot\)5H\(_2\)O, 2.67 mM MgSO\(_4\), 0.1 mM EDTA, 33.3 mM dithiothreitol, 40 or 100 units of RNase inhibitor (RNasin, Promega). Complementary DNA was then synthesized with 50 units of SuperScript RNase H\(^{-}\) reverse transcriptase (Life Technologies, Inc.) or with 100 units of Moloney murine leukemia virus reverse transcriptase (New England Biolabs). The products were analyzed on a 6% polyacrylamide sequencing gel.

Transfection and Luciferase Assay—Cells were grown in 6-well plates (well size 2.5 cm diameter, Falcon 3046) to a subconfluent state and transfected with plasmids by a liposome-mediated method (Transfection Reagent, DOTAP; Boehringer Mannheim Biochemicals). In each well, 2 \(\times\) 10\(^5\) cells were plated and grown overnight. The DNA/liposome mixture was prepared by mixing 0.1 \(\mu\)l of the DNA solution containing 3 \(\mu\)g of each plasmid DNA with 0.1 \(\mu\)l of the liposome solution (30 \(\mu\)l of DOTAP and 70 \(\mu\)l of serum-free medium). Cells were washed with serum-free medium and mixed with 0.2 \(\mu\)l of the DNA/liposome mixture, then incubated for 6 h at 37 \(^\circ\)C. Medium was then replenished with fresh culture medium, and cells were grown for an additional 36 h. Cells were washed with phosphate-buffered saline, then lysed in 100 \(\mu\)l lysis buffer, containing 1% Triton X-100, 25 mM Tris-phosphate, pH 7.8, 2 mM dithiothreitol, 2 mM EDTA, and 10% glycerol at room temperature for 10–15 min. The lysate was clarified by a brief centrifugation in a microcentrifuge and used for luciferase assay or stored at -70 \(^\circ\)C. For measurement of luciferase activity, 20 \(\mu\)l of lysate was mixed with 100 \(\mu\)l of Luciferase assay reagent (Promega) 1.07 mM Tricine, 1.07 mM (MgCO\(_3\))\(\cdot\)5H\(_2\)O, 2.67 mM MgSO\(_4\), 0.1 mM EDTA, 33.3 mM dithiothreitol, 270 \(\mu\)M coenzyme A, 470 \(\mu\)M luciferin, and 530 \(\mu\)M ATP. Luminescence was measured for 10 s in a Turner Designs Luminometer Model 20.

RESULTS

Sequence of the Exon/Intron Junctions and Sizes of Introns—Soh et al. (1994) prepared cosmid libraries from the 540-kb GART YAC and 150-kb fragmented YAC J18 (Cook et al., 1994) which were shown to contain the human IFN\(\gamma\)R2 gene. The cosmids were overlapped by genomic walking (Soh et al., 1994). Cosmids encompassing the IFN\(\gamma\)R2 gene were identified by hybridization to various regions of the cDNA (Fig. 1). EcoRI subfragments from the cosmids GC8-10 and GC3-59 shown to hybridize to Hu-IFN-\(\gamma\)-R2 cDNA were cloned into the EcoRI site of the plasmid pBS. Among the EcoRI fragments from the cosmid GC8-10, 14-kb, 10-kb, and 6-kb fragments hybridized to Hu-IFN-\(\gamma\)-R2 cDNA. However, none of these EcoRI fragments from the cosmid GC8-10 hybridized to oligonucleotide 6799, which was derived from the 5' region of the human IFN\(\gamma\)R2 gene (Fig. 1); therefore, GC8-10 did not contain the promoter. The 11-kb EcoRI fragment from the cosmid GC3-59 (plasmid GC3.59E11) hybridized to oligonucleotide 6799 indicating that the promoter was encompassed in this fragment. Together, both cosmids proved to encompass the entire IFN\(\gamma\)R2 gene.

All of the above EcoRI fragments were subcloned as smaller fragments and sequenced with oligonucleotide primers derived from various regions of the Hu-IFN-\(\gamma\)-R2 cDNA. The resulting sequences were analyzed to match the Hu-IFN-\(\gamma\)-R2 cDNA sequence to locate the exon/intron boundaries. The IFN\(\gamma\)R2 gene encoding the Hu-IFN-\(\gamma\)-R2 chain spans over 33 kb of genomic DNA and contains seven exons (Fig. 1). Exon 1 contains the 5'-untranslated region and part of the signal peptide. The signal peptide is encoded by exons 1 and 2, the extracellular domain by exons 2, 3, 4, 5, and 6. Exon 6 also encodes part of the extracellular domain, the entire transmembrane domain, and also part of the intracellular domain. Exon 7 encodes the remainder of the intracellular domain and contains the 3'-untranslated region. The sequences at the exon/intron boundaries are well conserved with respect to canonical acceptor/donor sites (AG/CT) except for the first intron boundary that contained a TG instead of GT (see Fig. 3).

Mapping the Transcriptional Initiation Sites of the Human IFN\(\gamma\)R2 Gene—Soh et al. (1994) reported two Hu-IFN-\(\gamma\)-R2 cDNA clones of different lengths. For the determination of the transcriptional initiation sites for both of these mRNAs and possibly others, primer extension analysis was performed. To examine start sites corresponding to the large cDNA clone pSK1, oligonucleotides 7513 (positions -157 to -140 of the genomic se-
structure of the gene for second chain of Hu-IFN-γ receptor.

The DNA sequence of a 1.25-kb XbaI fragment from clone GC3.59E11, which includes 371 bp of DNA upstream of the major transcriptional initiation site for the longer clone pSK1 and part of the first exon, was determined (Fig. 2). No consensus TATA boxes or CAAT boxes, sequence elements common to a variety of RNA polymerase II-regulated eukaryotic gene promoters, were identified within several hundred nucleotides upstream of the transcriptional initiation sites. Analysis of the Hu-IFN-γR2 promoter region showed that the first exon and 5'-flanking sequences (between nucleotides −1182 and +73) exhibited a high G + C content (64%). The G + C content of region immediately upstream of the ATG codon was much higher: between nucleotides −300 and −1, the G + C content was 84%. A computer-assisted search revealed a number of potential regulatory elements (Fig. 2), many of which are also present in the human and mouse IFN-γR1, and mouse IFN-γR2 (Ebenperger et al., 1995). A total of seven GC boxes representing potential binding sites for the transcription factor Sp1 (Dyman et al., 1985) were detected. Six of these sites were located upstream (positions −563, −213, −198, −188, −182, and −170) and one downstream (position −78) of the two groups of major transcription start sites for shorter transcripts. Two overlapping AP-2 sites (Imagawa et al., 1987) were located 79 and 80 nucleotides upstream (positions −728 and −727) of the end of the mRNA corresponding to clone pSK1. Two AP-2 sites were located 143 and 144 bp upstream (positions −257 and −256) of the beginning of the mRNA corresponding to the shorter clone pJS3. One AP-2 site was 19 bp downstream (position −94) of the beginning of the mRNA corresponding to the shorter clone pJS3. The mouse gene contains seven Sp1 sites and eight AP-2 sites in positions similar to those in the human sequence (Ebenperger et al., 1995). Conservation of transcription binding sequences among mouse and human IFN-γR2 promoters may imply a conserved function in the transcriptional regulation of these genes.

**Fig. 3. Exon/intron boundaries of the human IFNGR2 gene.** Exon and intron sequences are shown in upper- and lowercase letters, respectively. Nucleotides are numbered according to the cDNA sequence (ATG = 1). The nucleotide consensus sequences of the introns adjoining the splice junctions are shown in boldface.
Fig. 4. Localization of the transcriptional start sites of the human IFNGR2 gene by primer extension. Samples of total RNA (20 mg) from three cell lines were hybridized to the 5′-end-labeled oligonucleotide primer. After reaction with reverse transcriptase, the products were analyzed on a sequencing gel. The positions of the transcriptional start site are indicated by arrows. The molecular sizes were calculated by comparison with sequencing products from the same oligonucleotide primer. The numbers over each panel represent the nucleotide positions of the primers with respect to the A of the ATG translation start site taken as position 1. The sequence of each oligonucleotide of the Hu-IFN-γR2 cDNA in 3′ to 5′ direction is given under “Experimental Procedures” and summarized here with respect to the oligonucleotide number and its position, respectively: 6799, from nucleotide −73 to −93 upstream of the ATG translation start site; 7513, from nucleotide −140 to nucleotide −157; 7531 from nucleotide −310 to nucleotide −327; 7583 from nucleotide −529 to nucleotide −546; 7757 from nucleotide −752 to nucleotide −769.

Functional Analysis of the Human IFNGR2 Promoter—To study the promoter activity of the Hu-IFN-γR2 gene, a 1.2-kb fragment (positions −1182 to −27 in Fig. 2; Fig. 5) was inserted in both directions upstream of the firefly luciferase gene into plasmid pSR1 (see “Experimental Procedures”). The resulting plasmid was then transfected into HEp2 cells and assayed for luciferase activity. The plasmid containing the 1.2-kb fragment in the correct orientation gave high levels of luciferase activity. On the other hand, the plasmid containing the same fragment in the opposite orientation showed no luciferase activity. Little or no luciferase activity was observed with the pSR1 vector alone. Plasmid pSR2, a plasmid containing the luciferase gene under the control of the CMV promoter, was used as a positive control for luciferase activity. A larger 3.5-kb fragment, including the 1.2-kb fragment and 2.3 kb of upstream sequence, yielded identical results as the 1.2-kb fragment alone when inserted in the luciferase vector.

Deletion Analysis of the Promoter for the Human IFN-γR2 Gene—To analyze the importance of the putative cis-acting elements identified in the promoter region, a series of deletion mutants was tested for luciferase activity following transfection into HEp2 cells. The structure of the various deletion mutants and their relative promoter activities are shown in Fig. 5. The luciferase activity of the promoter construct containing the 1.2-kb fragment (pSR1-1.2) was chosen as a reference that was arbitrarily set at 100. Removal of the 5′ region from nucleotide −1182 to nucleotide −412 resulted in 66% retention of the promoter activity. Similarly, removal of nucleotide −545 to −27, which represented the 3′-half of the 1.2-kb fragment, resulted in retention of 57% of the activity. When the 5′ end (nucleotide −1182 to −1005) and 3′ end (nucleotide −410 to −27) were removed from the 1.2-kb fragment, only 27% of the promoter activity remained. Further truncation of the promoter region had a profound effect on promoter activity. Removal of most of the 5′ region, but retaining 83 bp (nucleotides −90 to −7) retained 31% of the promoter activity. By contrast, all of the promoter activity was lost when only the 30-bp fragment (nucleotides −37 to −7) was left. Virtually identical results were obtained in COS-7 cells (data not shown).

These data suggest that the elements located between positions −1182 and −37 play a role in the regulation of the IFNGR2 gene and are dispersed throughout this region corresponding to the AP-2 and Sp1 elements described. No significant positive elements are present upstream of nucleotide −1182.

DISCUSSION

In this report, we describe the structure of the IFNGR2 gene and its promoter region. In addition, we carried out a functional analysis of the promoter region. The major transcriptional initiation sites were identified and the sequence of the 5′ region of the gene was determined. An 83-bp fragment (−90 to −7) from this region could drive the expression of the luciferase gene in HEp2 cells at a level of activity equivalent to about one-third of the 1.2-kb promoter fragment.

Initiation of transcription on the human IFNGR2 gene occurs at multiple sites as established by primer extension assays. These transcription sites were similar among the cell lines examined indicating that Hu-IFN-γR2 mRNA is expressed in these different cells in a similar manner. Our finding of sets of initiation sites corresponding to the regions of the longer and shorter Hu-IFN-γR2 mRNAs is consistent with the Northern blots previously reported that showed two major classes of transcripts (Soh et al., 1994). Soh et al. (1994) reported that the shorter mRNA was predominant, suggesting that the initiation sites for the shorter transcript were dominant.

The fragment containing the promoter region is relatively rich in G + C (64–84%) and contains multiple GC boxes, but does not have typical TATA and CAAT boxes, a finding consistent with the presence of multiple initiation sites. Thus, the Hu-IFN-γR2 promoter has structural features common to the promoters for a number of housekeeping genes (Beck et al., 1990). As is typical of promoters without a TATA site and multiple origins of transcription (Ishii et al., 1986; Koh et al., 1993; Kaestner et al., 1994), Sp1 (GC boxes) binding sites are present in the promoter for IFNGR2. Within the human IFNGR2 promoter, potential regulatory elements such as Sp1 and AP-2 binding sites as well as other possible transcription
Fig. 5. Functional analysis of the human IFNGR2 promoter. On the top left, the approximate locations of the putative transcription factor binding sites are shown. Below, the structure of each construct is shown; the 5′ promoter fragment of each construct is shown as a shaded rectangular area, and the thin line represents the segment deleted. On the right, the corresponding promoter activities are shown as the relative intensity of the light generated by luciferase. Each construct was transfected into HEp2 cells, and the luciferase activity of the cell lysate was measured by luminance. The luciferase activity of the promoter construct containing the 1.2-kb fragment was chosen as a reference that was arbitrarily set at 100. The 1.2 fragment (positions −1182 to −27; Fig. 2) was obtained by digestion of an 11-kb EcoRI fragment from cosmID GC3-59 (Soh et al., 1994) with XbaI and DdeI and inserted upstream of the luciferase gene in plasmid pSR1. Smaller fragments shown in the figure were derived from the XbaI-DdeI region with restriction endonucleases as follows: the 638-bp fragment (−1182 to −545) with XbaI and HindIII; the 596-bp fragment (−1006 to −411) with SmalI; the 405-bp fragment (−411 to −7) with SmalI. These fragments were all inserted upstream of the luciferase gene in plasmid pSR1. The insertion of the 405-bp fragment into plasmid pSR1 led to plasmid pSR1-400 that contained an XhoI restriction endonuclease site at the 5′ end of the insert. Thus, to construct the plasmid pSR1-84 containing the 84-bp fragment (−90 to −7), plasmid pSR1-400 was digested with restriction endonucleases XhoI and NcoI, then both ends were filled in and recircularized by ligation. By this deletion, the fragment from nucleotide −406 to −91 was removed from plasmid pSR1-400, and only the fragment from nucleotide −90 to −7 was left in the resultant plasmid pSR1-84. To construct plasmid pSR1-31 containing the 31-bp fragment (−37 to −7), plasmid pSR1-400 was digested with restriction endonucleases XhoI and EagI; then both ends were filled in and recircularized by ligation. The basal luciferase plasmid without these promoter fragments (pSR1) and the luciferase construct with the CMV promoter were used as negative and positive controls, the bottom two constructs shown on the figure. The absolute values of the luciferase activities were as follows with respect to the relative values given in the figure: relative value of 100 was equivalent to 1,237 luminescence units; 57, equivalent to 740; 27, equivalent to 351; 13, equivalent to 852; 4, equivalent to 85; 31, equivalent to 407; 4, equivalent to 50; 13, equivalent to 171; and 1,500, equivalent to 20,000. These values were the average of duplicate determinations. The assays with these constructs were performed four times with similar results.

factor binding sites were present. For example, a TFIID-EIIa site, TATAAAA, is observed at position −1096 (Huang et al., 1988), and a Pu-box, GAGGA (Klemsz et al., 1990), at position −1090. Many Sp1 and AP-2 sites are also present in the mouse ifngr2 promoter.

The region with maximal promoter activity (−1182 to −37) contains seven Sp1 binding sites and five AP-2 binding sites. The elimination of the −544 to −27 fragment containing five Sp1 and three AP-2 sites reduced the promoter activity by a factor of 2, supporting a role for these elements in promoter activity of the IFNGR2 gene.

Overall, it appears that the functional elements are distributed over a wide range in the 1.2-kb fragment. No specific small region appears to account for the major activity. This is consistent with the multiple initiation sites for mRNA along this region.

Numerous viral and cellular genes have now been shown to contain Sp1 binding elements, and these are generally located 40–100 nucleotides upstream from the transcription start site (Kadonaga et al., 1987), a situation seen in the human IFNGR2 promoter. The transcription factor Sp1 has been shown to belong to a multigene family expressed ubiquitously. Other members of this family such as transcription factor Sp3 also bind to the Sp1 consensus GC box and exhibit binding activity similar to that of Sp1 (Kingsley and Winoto, 1992). Transcription factor AP-2 recognizes the SV40 enhancer as well as the regulatory regions of several cellular genes, including the human growth hormone, human c-myc, and histocompatibility H-2K\(^{\alpha}\) genes (Imagawa et al., 1987). Thus, our results suggest that transcription factor Sp1 or related proteins as well as transcription factor AP-2 are significant regulatory elements of the human IFNGR2 promoter.

Acknowledgments—We thank Ulrich Certa for the luciferase vectors and Eleanor Kells and Karen Manhine for assistance in preparing the manuscript.

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