Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 4774–4778, May 1992
Biochemistry

Behavior of a cloned murine interferon α/β receptor expressed in homospecific or heterospecific background

(cytokine receptor/PCR cloning/transfection)

GILLES UZÉ*, GEORGES LUTFALLA, MARIE-THÉRÈSE BANDU, DOMINIQUE PROUDHON†, AND KNUD E. MOGENSEN

Centre National de la Recherche Scientifique, Laboratoire d'Oncologie Virale, UPR 274, 94 801 Villejuif Cedex, France

Communicated by James E. Darnell, Jr., February 7, 1992

ABSTRACT **A murine interferon (IFN) α/β receptor was cloned from the IFN-sensitive L1210 cell line on the basis of its homology with the human receptor. A combination of methods that includes the screening of random-primed and oligo(dT)-primed cDNA libraries and polymerase chain reactions with a single-side specificity was used. At the amino acid level, the murine IFN-α/β shows 46% identity with its human counterpart. Both human WISH cells presenting a low sensitivity to mouse IFN and a murine L1210 mutant subline that does not express the receptor have been stably transfected with the murine IFN-α/β receptor. Whereas transfected human cells became sensitive to a limited number of mouse IFN-α/β subtypes, the transfected murine L1210 mutant was found to be fully complemented and became sensitive to all mouse IFN-α/β subtypes tested, including those that were not active on transfected human cells. These results strongly suggest that the receptor described here is implicated in the mediation of the activities of all murine IFN-α/β subtypes.**

The interferon (IFN) α/β family consists of not less than 20 related genes (1). In the mouse system, an IFN-α/β protein mixture is generally produced by cells infected by virus (2). The biological effects of these IFNs, including the inhibition of virus replication and the inhibition of cell growth, are mediated by a cell surface receptor (3, 4). In the murine system, the quantitative relationship between binding and biological activities of IFN-α/β has been only poorly studied; it is assumed that the general conclusions concerning the correlation between binding and activities of IFNs found in human (5–7) are also true for the murine system.

A cDNA encoding a human IFN-α/β receptor has already been cloned by an expression cloning strategy in which a mouse cell clone transfected with human DNA has been isolated on the basis of its acquired sensitivity to the human IFN-αB (8). This human IFN receptor is a 557-amino acid transmembrane protein. When this cDNA is expressed in mouse cells, transfected clones present binding sites for human IFN-αB on their surface and become proportionally sensitive to the antiviral activity of this particular IFN. However, transfected cells were poorly sensitive to other human IFN-α subspecies like IFN-αA and human IFN-β, at both binding and biological activity levels. These results suggest that the cloned receptor is not by itself sufficient to confer full sensitivity to human IFN-α/β subspecies and that, in this system of expression of the human receptor into a mouse recipient cell, high-affinity binding and biological response of the cell cannot be dissociated.

We report here the cloning from the mouse L1210 cell line of a murine counterpart of the cloned human IFN-α/β receptor and its sequences.‡ We show that the mutation of the IFN-resistant L1210 R101 subline, which does not express the receptor (3, 9), affects the steady-state level of the receptor transcript and that this mutation is fully complemented by the expression of the cloned receptor.

MATERIALS AND METHODS

IFNs. Natural mouse IFN-α/β was produced by C243 cells induced by Newcastle disease virus and partially purified as described (10). Recombinant mouse IFN-α8 and IFN-α11 were a generous gift from G. Vodjdani (Centre National de la Recherche Scientifique, Villejuif, France). They were produced by transfected simian COS-7 cells (11, 12). Relative antiviral titers were estimated by a cytopathic inhibition assay using vesicular stomatitis virus or encephalomyocarditis virus as challenge virus (5, 8).

Cell Lines and Transfections. Murine L cells were grown in BSC medium [Eagle's minimum essential medium/glucose (3.5 g/liter)/tryptose phosphate (2.95 g/liter)], containing 10% (vol/vol) newborn calf serum. Human WISH cells were grown in BSC medium containing 10% (vol/vol) fetal calf serum. They were transfected by the calcium phosphate precipitation technique (13) and selected in medium containing G418 (GIBCO) at 1.25 mg/ml. The IFN-sensitive and -resistant mouse L1210 cell lines (9) have been recloned from the sensitive and resistant clones used by Aguet (3) and named S61 and R101, respectively. They were cultivated in RPMI 1640 medium supplemented with 10% fetal calf serum, transfected by the electroporation procedure (14) in a 0.4-cm diameter cuvette (300 V; 960 μF) with a Bio-Rad Gene Pulser, and selected with G418 at 1.5 mg/ml.

General Methods. Standard procedures were as described (15). All DNA fragments were cloned into Bluescript KS II+ or SK II− plasmids from Stratagene or into the mammalian expression vector pVADN1. pVADN1 is derived from pAB10 (8) but it contains the neomycine-resistance selection cassette from pSV2neo (16). RNA was prepared according to Chomczynski and Sacchi (17). DNA sequencing and handling were as described (18).

Genomic and cDNA Libraries. NIH 3T3 DNA genomic library in λ FIX II was from Stratagene. Screening with the human probe 3E7H3 (8) was performed under low-stringency conditions according to Howley *et al.* (19). Briefly, hybridization was at 42°C in 1 M NaCl/0.1% Ficoll/0.1% polyvinylpyrrolidone/0.1% bovine serum albumin/5% dextran sulfate/5 mM EDTA/1% SDS/25% (vol/vol) deionized formamide in 50 mM sodium phosphate (pH 7.2). Washing was at 42°C in 1 M NaCl/5 mM EDTA/1% SDS/30% formamide in 50 mM sodium phosphate (pH 7.2).

Abbreviations: IFN, interferon; nt, nucleotide(s).

*To whom reprint requests should be addressed.
†Present address: Laboratoire de Biologie Moléculaire Végétale, Université Joseph Fourier, BP 53X, 38 041 Grenoble Cedex, France.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. M89647).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Oligo(dT)- or random-primed cDNA libraries were constructed in the λ ZAPII phage vector (Stratagene) from poly(A)$^+$ RNA of L1210 S61 cells using the cDNA synthesis system from Amersham. Packaging reactions were done using Gigapack gold extracts (Stratagene). cDNA libraries were screened under high-stringency condition according to Church and Gilbert (20) with the probes indicated in the text.

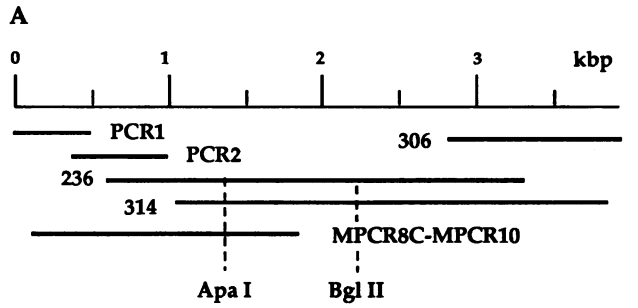
PCR Technologies. PCRs were performed in a DNA thermal cycler from Perkin–Elmer/Cetus using *Taq* DNA polymerase in the buffer recommended by Cetus. The following oligonucleotides indicated in the text were used: RIC10 (5′-GGAATTCCCCCCCCCC-3′; *Eco*RI site underlined), BamC10 (5′-CGGGATCCCCCCCCCC-3′; *Bam*HI site underlined), MPCR2 (5′-GGAATTCCCGTGTAGACAG-TATCTTGAG-3′, cDNA positions 999–978; *Eco*RI site underlined), MPCR4 (5′-GCGGATCCTCATTCCACGAA-GATGTGC-3′, cDNA positions 443–423; *Bam*HI site underlined), MPCR8C (5′-CCAGGATCCGAAGGAG-GAGAATGTGAGC-3′, cDNA positions 54–72; *Bam*HI site underlined), MPCR10 (5′-CGGGATCCGCAGAAGCTG-GCAGCCAC-3′, cDNA positions 1937–1920; *Bam*HI site underlined).

The cloning of the 5′ part of the cDNA from L1210 S61 poly(A)$^+$ RNA by PCR with a single-sided specificity was as follows: For the isolation of fragment PCR2, the reverse transcription was initiated on 1 μg of RNA with 1 μM of an oligonucleotide from positions 1029 to 1010 in a 20-μl reaction mixture containing all four dNTPs (each at 0.5 mM), 1 unit of RNase Block II (Stratagene), and 200 units of murine Moloney leukemia virus reverse transcriptase (BRL) in the buffer supplied by BRL. First-strand cDNAs were recovered by chromatography on Sepharose CL6/B (Pharmacia) and tailed with dG in a 100-μl reaction mixture containing 0.1 mM dGTP, 20 units of terminal deoxynucleotidyltransferase (Stratagene), and the buffer supplied by Stratagene. Tailed cDNAs were recovered by chromatography on Sepharose CL6/B and amplified using RIC10 and MPCR2 primers (30 cycles: 1 min at 94°C, 2 min at 50°C, 3 min at 72°C). Amplified material was cloned as *Eco*RI fragments. For the isolation of PCR1 fragment, the same procedure was used except that the reverse transcription was initiated with an oligonucleotide from positions 964 to 941. The PCR was performed with BamC10 and MPCR4 (30 cycles: 1 min at 94°C, 2 min at 60°C, 3 min at 72°C) and cloned as *Bam*HI fragments.

For the amplification of cDNAs containing the open reading frame sequence, the reverse transcription reaction of L1210 S61 poly(A)$^+$ RNA was primed with an oligonucleotide from positions 2612 to 2594. These cDNAs were then amplified by a PCR using MPCR8C and MPCR10 primers (30 cycles: 1 min at 94°C, 2 min at 60°C, 4 min at 72°C) and cloned as *Bam*HI fragments.

RESULTS

Isolation of Murine IFN-α Receptor-Encoding Sequences. The choice of the human IFN-α receptor probe and its hybridization condition to its murine DNA counterpart were first determined on genomic murine DNA Southern blots. A murine IFN-α receptor cDNA was then cloned in six steps. The five overlapping cDNA fragments are represented in Fig. 1*A*. In the first step a murine NIH 3T3 cell line genomic DNA library in a λ phage was screened by the human probe 3E7H3 (8). One exon of a murine IFN α receptor gene was identified in a 775-base-pair (bp) *Sau*3A subfragment of a λ phage insert by comparison of its sequence with the sequence of the human probe. This exon (which in further analysis appeared to be altered during the construction of the library) consisted of nucleotides (nt) 913–1075 of the cDNA sequence (Fig. 1*B*). The sequence of this coding fragment was used to design oligonucleotides to clone the 5′ part of the cDNA from L1210 S61 cell line RNA by the PCR with a single-sided specificity.



B

```
GCGGAGGCCTAGCTGCCCAGAGGTAGTCTCCAGCTCCGCGGTGCTGCTGAGGAGAAGGAG 60
GAGAATGTGAGCCGCCGCCCGGCCTCCCAAGACGATGCTCGCTGTCGTGGGCGCGGCGGC 120
CCTGGTGCTGGTGGCCGGGGCGCCTTGGGTGCTACCCTCAGCTGCAGGTGGAGAAAATCT 180
GAAACCTCCTGAGAATATAGACGTCTACATTATAGATGACAACTACACCCTAAAGTGGAG 240
CAGCCACGGAGAGTCAATGGGCAGTGTGACCTTTTCAGCAGAATATCGAACAAAAGACGA 300
GGCGAAGTGGTTAAAAGTGCCTGAATGTCAACATACTACAACGACCAAGTGTGAATTCTC 360
TTTACTGGACACAAATGTGTATATCAAAACACAGTTTCGTGTCAGAGCAGAGGAAGGGAA 420
CAGCACATCTTCGTGGAATGAGGTTGATCCGTTTATTCCATTCTACACAGCTCACATGAG 480
CCCCCCAGAAGTACGTTTAGAAGCTGAAGATAAAGCCATACTAGTCCACATCTCTCCTCC 540
CGGACAAGACGGGAACATGTGGGCACTGGAGAAACCTTCCTTCAGTTACACCATACGAAT 600
CTGGCAGAAGTCTTCCAGTGACAAAAAAACTATTAACTCTACGTATTATGTAGAAAAGAT 660
ACCAGAACTCTTGCCAGAGACTACTTACTGTTTAGAAGTTAAAGCAATACATCCGTCACT 720
TAAGAAACACAGCAATTACAGCACTGTGCAGTGTATAAGCACCACAGTGGCAAATAAAAT 780
GCCTGTGCCAGGAAATCTCCAAGTGGATGCCCAAGGCAAGAGCTATGTCCTGAAATGGGA 840
CTACATTGCGTCTGCAGACGTGCTCTTCAGGGCACAGTGGCTTCCTGGCTATTCAAAAAG 900
CAGTTCTGGAAGCCATTCAGATAAATGGAAACCAATACCAACCTGTGCAAATGTCCAGAC 960
TACGCACTGTGTCTTTTCTCAAGATACTGTCTACACAGGAACGTTCTTTCTCCATGTACA 1020
AGCCTCAGAGGGAAATCACACATCCTCTTTGGTCTGAAAGAAGTTTATTGATTCTCAAAA 1080
ACACATTCTCCCTCCTCCTCCGGTCATTACTGTCACCGCCATGAGTGACACCTTGCTTGT 1140
TTATGTCAACTGTCAGGACAGCACATGTGATGGACTCAATTACGAAATCATCTTTTGGGA 1200
AAACACTTCCAATACTAAGATAAGCATGGAGAAGGATGGCCCAGAGTTCACCCTCAAGAA 1260
CCTGCAGCCGCTGACTGTGTACTGTGTCCAGGCCAGAGTGCTCTTCAGGGCCCTGCTGAA 1320
TAAGACCAGCAACTTCAGTGAAAAGCTGTGTGAGAAAACACGTCCAGGAAGTTTTTCCAC 1380
GATCTGGATTATAACTGGATTAGGTGTTGTGTTCTTCTCTGTCATGGTCCTTTATGCTTT 1440
GAGGAGCGTCTGGAAATACCTGTGTCATGTGTGCTTCCCACCACTCAAGCCTCCCCGCAG 1500
TATTGATGAGTTTTTCTCTGAGCCGCCTTCAAAAAACCTTGTACTTCTGACGGCTGAGGA 1560
GCACACGGAAAGATGCTTCATCATTGAGAATACAGACACGGTCGCTGTAGAAGTAAAGCA 1620
CGCGCCTGAGGAGGACCTCAGGAAGTACAGCTCACAGACCAGCCAGGACTCGGGCAACTA 1680
TTCCAACGAAGAGGAGGAGAGTGTGGGCACCGAGAGCGGCCAAGCTGTGCTCTCCAAAGC 1740
TCCCTGCGGGGGGCCATGCAGCGTGCCTAGCCCTCCTGGGACCTTGGAAGACGGGACCTG 1800
CTTCCTGGGAAATGAAAAATATCTTCAGAGCCCAGCCCTGAGGACAGAGCCAGCTCTTCT 1860
CTGCTGACAGGTGCTCGGCCCAGGCTGGGGGCCGTGTGGAGGAGACAGACTTATCTGAAG 1920
TGGCTGCCAGCTTCTGCTGCCAGCTGAAGCCCTGGGTGCTGTTTGTGCCACAGATGAGG 1980
CTCACTGATGTCAGGCATCTGGTGGTTCTGTCTCGGTGTTTGTCCTCTAGGTGACATTTA 2040
GAACATTCCGATTTTTAATCCTGCCGTAGCCCCTCAGTGTGTCACAGGTACCATTGTCTA 2100
CAGCCGCGCTTCCTAACCCATACCTCACCACAGCCGTGGCTTCTGACCAGAAGTCACTGA 2160
AACCATGGAGAAAGGGGACACTAACTGGAGGAGCTGGCGTTGTTTAAGATCTCAGTGTGG 2220
CCTGGGTGTGCCCGTTGTACTCTAGTACTTGCAGAACTAGAAAATGCCAGGGACAAAAAG 2280
CAGGCATGAACCATTCAGTAAGAGAGAAGCTAGTGAACCTGATGGTGAAAGAAGACCGTG 2340
TCCAGTCAGTGTTTGTGTGAAGGCCCTGTGCTGCTCTCTGTAGCCTTCCCGTGGTACTTA 2400
TAAAAACCAGGCCGAACCCTGTCCTCTGTCCGGCACTATTCAGGTCCCTTCCCAGCTCCA 2460
GGTACTTAAAAAACAAAGTGTTGGGTCCTGGGGTGAGGGAACAGCTTCGACGGCACAGTG 2520
CTGCCTGCAGCGCCCTATAAGTGACCTTAGATGAATGCTCCATGAGCATCTTCGGTAGGC 2580
AGGACATCCAGCAGTGCTGGTGGCTCCAGGGCAGACTCAGTTGCAGGCCAAAAAGGAAGA 2640
GAGAGGAGTCATAGCCCTGCCCCAATCGAGCTTGTTGCTCGCTGCACTGCGCCTTTGTAA 2700
TCAATCTTGGTTTTTTTGTTTTGTTTTGTTTTTTTAACTTTTAGGTGATAACATATTCT 2760
CAGATATAGCACACAAGAGACACTACACAAATTACGTCTGAAAGTTTGATAGAAAAGAG 2820
CTTTTGACTGGGGTAGACAGCTTGCTCCTCACTACTACCTTGTATCTCAGTCTCCTCAAC 2880
AGAGAGAGAATGCAGATTGATTTTCATCCTGAGTTTTATTTTCTTGCCCGGTTTCTGGGT 2940
TCGGGTGTGCTTTCAAGGCTCTGAACAGCTTGTCTGCCCACAGGGGAGCCCGGAGACCCA 3000
GCCTTTTGGCTAAATGTTGCAGGTTCCTGTACTTGAAGGACTCCAGCTCCTCTGTAGAGG 3060
GCATGGTCATCGCGGTCTGCAATCACGCTGATACCCAGAAAAATGGCCACACAACTCAAA 3120
TCTGGTGCCACGCTTTAATAATTCTACCCAGCACGGCCCAGCCCTGTTCACGGCGGAAAA 3180
GCTCCTTCTGCCAGGACAGCCTGTGAAGAAACAAGACCTCCGAGGACGTGGCCAAGGAAG 3240
GCCCAGATGCTAGCGGGAAGTTGTTAGTTGGCACTCTTTCATCTCTCAACAACTGTGACA 3300
CAGGTCTTCAGGGTGAAGCCCAAAGCAGCTATCGGTTGCAGCAGCGGGTAAGGAGGGGGC 3360
TTCACCTGAGCCTGCCCTTCTGGGCGAGCAGTTCTACCTGCACACTTCAAGACAGCCTCC 3420
ATGACCCTTTTCACGGCACACCGCCTGCTTTTGGTGTGACCGGGGTTTCCTGTGCAGGCA 3480
CATGTTCTTAAGGAATGCCATGCACACAGGCCTGAAGAGCTGGCTCAGCGGGTAGAGCAC 3540
AGGTTGCTCTTGTCTGGGATCTGAGCTCTGTCCCCAGCACCCACAGAGTGCTTCTGGTTG 3600
CCTCAGACTCCAGGGGTGCACAAGGCCATGTAAGCAAAGACATGTAAGCAAAGACAGTTG 3660
TACACAGAGAAGTCAAACCTTTTTGAAAAAAAGAAACACCACGGGCTGGAGAGATGGCTC 3720
AGTGGTTAAGAACACTGACCACTCTTCCAGAGGTCCTGAGTTCAATTCCCAGCAACCACA 3780
TGGTGGCTCACAACCATCTGTAATAGGATCTGATGCCCCCTTCTGGTGTGTCTGAAGATA 3840
GCTACAGTGTATTCATATATAAAATAATAAATACATCCTTTTTTTTCTTTTTTTAAAAAA 3900
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA                    3942
```

FIG. 1. Structure and sequence of the cDNA clones of the murine IFN-α/β receptor. (*A*) Schematic diagram representation of the various overlapping cDNAs. The *Apa* I and *Bgl* II restriction sites used for the reconstruction of the coding sequence are indicated. (*B*) Complete nucleotide sequence of the murine IFN-α/β receptor cDNA. The open reading frame is underlined. The restriction sites cited in the text are boxed. From 5′ to 3′, they are *Apa* I in the coding sequence, *Pvu* II, *Bgl* II, *Pst* I, and *Bgl* I in the noncoding sequence.

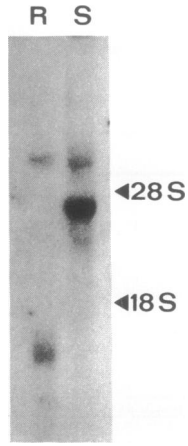


FIG. 2. Northern blot analysis of 5 μg of oligo(dT)-selected RNA from wild-type murine L1210 S61 clone (lane S) or IFN-resistant murine L1210 R101 clone (lane R) hybridized with murine IFN-α/β receptor cDNA (1884-bp MPCR8C–MPCR10 PCR fragment). rRNA mobilities are indicated as 28 S and 18 S.

Two fragments were obtained: the first (PCR2, nt 353–999 from the *Eco*RI site of the cDNA) was from an *Eco*RI cloning of the PCR product between the oligonucleotides RIC10 and MPCR2, and the second (PCR1, nt 1–443) was from a *Bam*HI cloning of the PCR product between the oligonucleotides BamC10 and MPCR4. cDNA 236 (nt 594–3106) was isolated in a random-primed cDNA library from the L1210 S61 cell line with the fragment PCR2 as a specific murine probe. cDNA 314 (nt 999–3751) was isolated from the same random-primed cDNA library with the 585-bp *Pvu* II–*Pst* I fragment of cDNA 236 (nt 1941–2525). cDNA 306 (nt 2791–3942) was isolated from an oligo(dT)-primed cDNA library from L1210 S61 cell line with the 377-bp 3′ *Bgl* I fragment of cDNA 314 (nt 3375–3751).

Fig. 1*B* represents the 3942-bp nucleotide sequence of the five overlapping cDNA cloned fragments. This reconstituted cDNA sequence displays an open reading frame of 1770 bp closed in both 5′ and 3′ by stop codons. A single DNA fragment containing the whole open reading frame was synthetized by a PCR between the oligonucleotides MPCR8C and MPCR10 and cloned as a 1884-bp *Bam*HI fragment in Bluescript KS II+. Two such fragments were entirely sequenced to ascertain the sequence presented in Fig. 1*B*, especially to check the sequence of nt 54–593, which had been determined only by sequencing cDNA fragments amplified by PCR.

As shown in Fig. 2, the 1884-bp MPCR8C–MPCR10 DNA fragment detects an ≈4-kbp predominant transcript from the murine L1210 S61 cell lines but not from the IFN-α/β-resistant murine L1210 R101 cells, which have been described as lacking the IFN-α/β receptor (3, 9). The human and murine open reading frame present 63% identity. The murine 3′ untranslated region is 2 kilobases long and cannot be aligned with the human 3′ untranslated region that consists mainly of an *Alu* repeat.

Structure of the Murine IFN-α/β Receptor. The 590-amino acid (M_r, 65,734) polypeptide sequence presumably encoded by the murine IFN-α/β receptor cDNA is shown in Fig. 3 and murine and human IFN-α/β receptor sequences are compared. This alignment, which imposes a 7-amino acid gap in the mouse receptor, has been obtained by the CLUSTAL program (21).

At the amino acid level, the murine IFN-α/β receptor presents 46% homology with the human receptor. Hydropathy plots (22) predict a putative signal peptide of 26 amino acids and a single transmembrane domain. The N-terminal

```
MOUSE MLAVV-GAAALVLVAGAPWVLPSAAGGENLKPPENIDVYIIDDNYTLKWSSHGESMGSVTFSAEY 64
HUMAN MMVVLLGATTLVLVAVAPWVLSAAAGGKNLKSPQKVEVDIIDDNFILRWNRSDESVGNVTFSFDY 65
      *  *  **  ***** *****  **** *** *    * *****  * *    ** * ****  *

MOUSE RTKDEAKWLKVPECQHTTTTKCEFSLLDTNVYIKTQFRVRAEEGNSTSSWNEVDPFIPFYTAHMS 129
HUMAN QKTGMDNWIKLSGCQNITSTKCNFSSLKLNVYEEIKLRIRAEK-ENTSSWYEVDSFTPFRKAQIG 129
             * *   **  * *** ** *  ***     * ***    **** *** * **  *

MOUSE PPEVRLEAEDKAILVHISPPGQDGNMWALEKPSFSYTIRIWQKSSSDKKTINSTYYVEKIPELLP 194
HUMAN PPEVHLEAEDKAIVIHISPGTKDSVMWALDGLSFTYSLLIWKNSSGVEERIENIYSRHKIYKLSP 194
      **** ********  ****   *  ****   ** *   **  **     *   *   **  * *

MOUSE ETTYCLEVKAIHPSLKKHSNYSTVQCISTTVANKMPVPGNLQVDAQGKSYVLKWDYIASADVLFR 259
HUMAN ETTYCLKVKAALLTSWKIGVYSPVHCIKTTVENELPPPENIEVSVQNQNYVLKWDY-TYANMTFQ 258
      ****** ***      *   ** * ** *** *  * * *  *  *   *******   *   *

MOUSE AQWLPGYSKSSSGSHSDKWKPIPTCANVQTTHCVFSQDTVYTGTFFLHVQASEGNHTSFWSEEKF 324
HUMAN VQWLHAFLKRNPGNHLYKWKQIPDCENVKTTQCVFPQNVFQKGIYLLRVQASDGNNTSFWSEEIK 323
       ***    *   * *  *** ** * ** ** *** *     *   * **** ** *******

MOUSE IDSQKHILPPPPVITVTAMSDTLLVYVNCQDSTCDG-------LNYEIIFWENTSNTKISMEKDG 382
HUMAN FDTEIQAFLLPPVFNIRSLSDSFHIYIGAPKQSGNTPVIQDYPLIYEIIFWENTSNAERKIIEKK 388
       *        ***      **    *                  * ***********

MOUSE PEFTLKNLQPLTVYCVQARVL-FRALLNKTSNFSEKLCEKTRPGSFSTIWIITGLGVVFFSV-MV 445
HUMAN TDVTVPNLKPLTVYCVKARAHTMDEKLNKSSVFSDAVCEKTKPGNTSKIWLIVGICIALFALPFV 453
          *  ** ******* **       *** * **   **** **  * ** * *     *    *

MOUSE LYALRSVWKYLCHVCFPPLKPPRSIDEFFSEPPSKNLVLLTAEEHTERCFIIENTDTVA-VEVKH 509
HUMAN IYAAKVFLRCINYVFFPSLKPSSSIDEYFSEQPLKNLLLSTSEEQIEKCFIIENISTIATVEETN 518
       **          * ** ***  **** *** * *** * * **  * ******  * * **

MOUSE APEEDLRKYSSQTSQDSGNYSNEEEESVGTESGQAVLSKAPCGGPCSVPSPPGTLEDGTCFLGNE 574
HUMAN QTDEDHKKYSSQTSQDSGNYSNEDESESKTSEELQQDFV-------------------------- 557
          **  ***************** *    *

MOUSE KYLQSPALRTEPALLC                                                  590
HUMAN ----------------
```

FIG. 3. Comparison of the predicted amino acid sequence of the murine IFN-α/β receptor to that of the human IFN-α/β receptor from ref. 8. Hydrophobic regions are in boldface type, potential sites of N-linked glycosylation are underlined, and identities are indicated by stars.

Table 1. Antiviral titers of IFN preparation assayed on parental human WISH cells, transfected WISH WC22 clone, or murine L cells

	IFN, units/ml			Ratio	
	WISH	WC22	L	WC22/ WISH	L/ WC22
Human IFN-α	2344	4,570	—	2	—
Mouse IFN-α/β	676	263,026	4,786,300	389	18
Mouse IFN-α8	<20	76	15,848	>4	209
Mouse IFN-α11	1259	245,470	794,328	195	3

Ratios are intended to be compared in a vertical sense (between IFNs) only.

potential extracellular part of the murine receptor contains ≈400 amino acids and presents an internal symmetry similar to that described for its human counterpart (23). The stretch of three prolines that separates the two domains of the extracellular part of the human receptor is replaced by Pro-Val-Pro on the mouse receptor.

The 141-amino acid putative intracytoplasmic portion of the mouse receptor is 41 amino acids longer than its human counterpart. This 41-amino acid sequence does not present significant similarity to known protein sequences as checked by computer-assisted searches in the National Biomedical Research Foundation data base (Release 28). Like its human counterpart, the intracytoplasmic part of the murine receptor appears to be remarkably acidic.

The murine IFN-α/β receptor exhibits eight potential N-linked glycosylation sites; five are in homologous positions compared to the human, including one in the intracellular part.

Expression of the Murine IFN-α/β Receptor cDNA. The two sequenced coding-phase-containing cDNA fragments synthesized by a PCR between MPCR8C and MPCR10 oligonucleotides contain purine transitions probably introduced by the *Taq* DNA polymerase during the PCR process. These mutations introduce amino acid substitutions: the first fragment codes for a receptor with an Asn → Asp substitution at amino acid position 499, and the second fragment codes for a receptor with a Val → Met substitution at position 256 and an Asn → Ser substitution at position 528. An unmutated DNA fragment covering the coding sequence of the cDNA was, therefore, reconstructed by ligating the 1.3-kbp 5′ *Apa* I fragment (nt 54–1309 or amino acids 1–406) of the first MPCR8C–MPCR10 PCR-synthetized cDNA fragment with the 0.9-kbp *Apa* I–*Bgl* II fragment of cDNA 236 (nt 1310–2208 or amino acids 407–590). This 2155-bp fragment was introduced immediately downstream of the adenovirus 2 major late promoter from the mammalian expression vector pVADN1 to obtain pVADN1csr.

To investigate the functionality of the cloned mouse IFN receptor, we have transfected pVADN1csr into the human WISH cell line. Table 1 shows the antiviral titer of various mouse IFN-α/β subtypes assayed on parental human WISH cells, on the representative WC22 clone stably transfected with pVADN1csr, or on the murine L cell line. The transfected WC22 clone manifests a 389-fold increase in sensitivity to the natural IFN-α/β induced in mouse C243 cells by Newcastle disease virus, and a 195-fold increase in sensitivity to the recombinant mouse IFN-α11 subtype. Interestingly, Table 1 also shows that the recombinant mouse IFN-α8 presents only a low activity on the transfected WC22 clone. This discrepancy between the capacity of mouse IFN subspecies to induce an antiviral state in a human cell expressing the murine receptor is illustrated by the ratio of the IFN titer measured on the murine L cell line to the IFN titer measured on the WC22 clone. These ratios indicate that the murine receptor expressed in human WISH cells is 70 times less active (209/3) with mouse IFN-α8 than with mouse IFN-α11. It was thus interesting to express the mouse receptor encoded by pVADN1csr in a mouse background represented by the IFN-resistant L1210 R101 clone, which does not express the receptor at the binding activity level (3) nor at the RNA level (see Fig. 2), to see if the cloned mouse receptor can transduce the signal given by the mouse IFN-α8 when expressed in a mouse background. The behavior of one transfected cell clone called RC17 was compared with the parental R101 clone or with the IFN-sensitive L1210 S61 clone for some biological activities of various mouse IFN-α and -β subtypes. Fig. 4 shows that the R101 clone is of course IFN-resistant but that the transfected RC17 clone is as sensitive to the antiproliferative activity of natural IFN-α/β as the wild-type S61 clone. The same results have been obtained with recombinant IFN-α8 and -α11 (data not shown). We also tested the sensitivity of the RC17 clone for the antiviral activity of mouse IFN-α and -β. Fig. 5 shows the similar behavior of RC17 and wild-type S61 clone for the antiviral activity of natural mouse IFN-α/β and recombinant IFN-α8 and -α11.

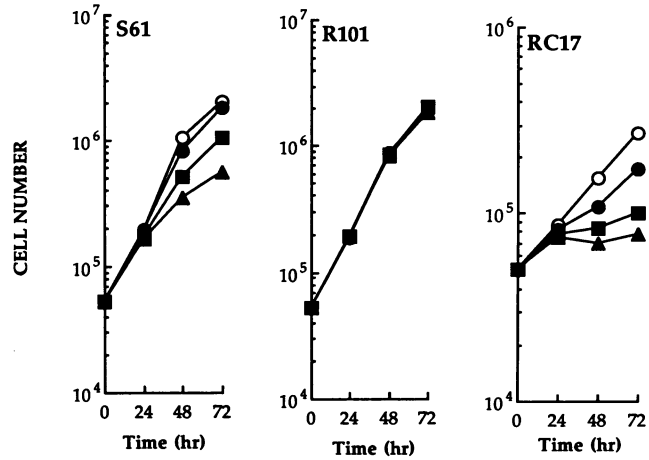


FIG. 4. Growth curves of the wild-type L1210 S61 clone (S61), the IFN-resistant L1210 R101 clone (R101), or the IFN-resistant L1210 R101 clone transfected with the murine IFN-α/β receptor cDNA (RC17) without (open symbols) or with (solid symbols) natural murine IFN-α/β at 10 units/ml (circles), 100 units/ml (squares), or 1000 units/ml (triangles).

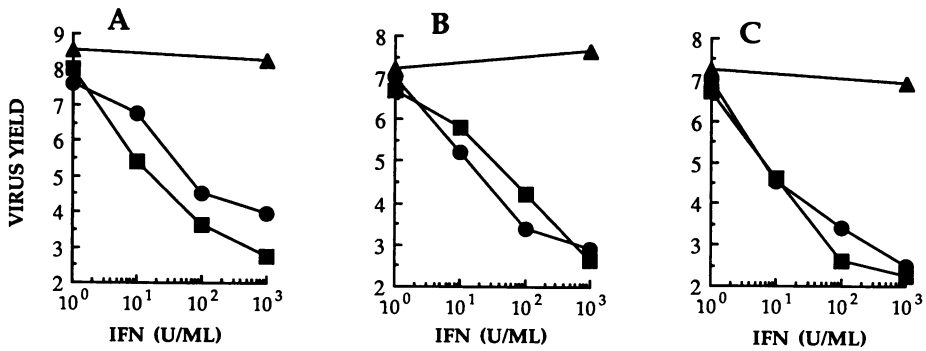


FIG. 5. Production of vesicular stomatitis virus by the wild-type L1210 S61 clone (circles), the IFN-resistant L1210 R101 clone (triangles), or the IFN-resistant L1210 R101 clone transfected with the murine IFN-α/β receptor cDNA (RC17 clone in squares) treated with natural mouse IFN-α/β (*A*), recombinant mouse IFN-α8 (*B*), or -α11 (*C*). U, unit(s).

These results show that the mutation that affects the expression of the receptor in the L1210 R101 clone is fully complemented by the cloned receptor. Consequently, we can say that the cloned receptor is necessary for mediating the activities of all tested mouse IFN-alpha subspecies, including those that present a low activity when the receptor is expressed in a human background.

DISCUSSION

In this report, we document the structure of the murine counterpart of the human IFN-α/β receptor. The 3942-bp cDNA sequence reported here corresponds to the ≈4-kbp major transcript detected in the pre-B L1210 S61 cell line but also in all IFN-sensitive mouse cell lines tested, including the hepatoma BTG 9A cell line or the L cell line (data not shown). This cDNA was cloned from L1210 S61 poly(A)$^+$ RNA by a combination of methods that include screening of random-primed and oligo(dT)-primed cDNA libraries and PCR technology. The major difference between the human and the murine mRNA is in their size since the murine transcript is 1.2 kbp longer than its human counterpart; however, the protein encoded by the murine cDNA has a putative intracytoplasmic region 41 amino acids longer than the human receptor.

The overall organization of the murine and human IFN-α/β receptors appears remarkably similar. Like the human receptor, the putative extracellular part of the murine IFN-α/β receptor appears to be also organized in two domains of ≈200 amino acids presenting 27% identity. It is thus likely that the mouse receptor belongs also to the cytokine receptor family identified by a predicted immunoglobulin-like folding of the extracellular parts (24, 25). Furthermore, the intron–exon organization of the gene encoding the murine IFN-α/β receptor (data not shown) matches with the evolutionary model we have proposed (18) for the cytokine receptor gene family.

When the mouse receptor is expressed into human WISH cells, transfected cells become sensitive to recombinant mouse IFN-α11 and to natural mouse IFN-α/β in which IFN-β represents ≈80% of the total IFN (2, 26, 27). However, those cells present only a low sensitivity to recombinant mouse IFN-α8. This situation is reminiscent of the behavior of mouse cells transfected with the human receptor that are mainly sensitive to human IFN-αB and not to the other human IFN-α or -β subspecies (8).

There are thus two kinds of models to explain these discrepancies: the first involves the existence of different receptors for the different IFN-α/β subspecies, and the second, which has been suggested (8, 28), involves only one receptor system but includes the existence of another level of regulation of the activity of IFN. This level of regulation would consist in one or several factors cooperating with the cloned receptor to form a fully functional receptor complex. To check that point, we have taken advantage of the IFN-resistant mouse L1210 R101 cell subline, which has been described as a totally IFN-resistant mutant that does not express the receptor (3, 9), to express the cloned murine receptor in its normal environment. This cell line shows a structurally altered IFN-α/β receptor gene (data not shown). In a first step, we have shown that the resistant clone does not express the receptor transcript found in the sensitive clone. We have then transfected the IFN-resistant mouse L1210 R101 clone with the mouse receptor. The behavior of the transfected resistant clone has been found to be similar to that of the sensitive clone for the antiviral and antiproliferative activities of natural mouse IFN-α/β or of all recombinant mouse IFN-α subspecies tested. This includes the mouse IFN-α8 which is the IFN-α subspecies that is poorly active when the murine receptor is expressed in the heterologous background of human cells. The complementation of the L1210 R101 clone by the mouse receptor cDNA clearly demonstrates that the receptor described here is implicated in the activities of probably all IFN-α subspecies including IFN-β. Like for most of the receptors belonging to the cytokine receptor family in which the need of at least two proteins to form a functional receptor appears to be a general model (29), it is likely that the IFN-α/β receptor works with (an) other factor(s) also implicated in the regulation of the activities of the IFN subspecies.

We thank I. Gresser for his support and U. Hibner for helpful discussions. We thank G. Vodjdani and J. Doly for the gift of recombinant mouse IFNs. This work was supported in part by grants from the Direction des Recherches Etudes et Techniques (89.34.132), the Agence National de la Valorisation de la Recherche (A8906303Q), and the Association pour la Recherche sur le Cancer.

1. Weissmann, C. & Weber, H. (1986) *Prog. Nucleic Acid Res. Mol. Biol.* **33**, 251–300.
2. Kawade, Y., Aguet, M. & Tovey, M. G. (1982) *Antiviral Res.* **2**, 155–159.
3. Aguet, M. (1980) *Nature (London)* **284**, 459–461.
4. Mogensen, K. E., Uzé, G. & Eid, P. (1989) *Experientia* **45**, 500–508.
5. Mogensen, K. E. & Bandu, M.-T. (1983) *Eur. J. Biochem.* **134**, 355–364.
6. Uzé, G., Mogensen, K. E. & Aguet, M. (1985) *EMBO J.* **4**, 65–70.
7. Eid, P., Bandu, M.-T., Uzé, G. & Mogensen, K. E. (1988) *Eur. J. Biochem.* **171**, 675–682.
8. Uzé, G., Lutfalla, G. & Gresser, I. (1990) *Cell* **60**, 225–234.
9. Gresser, I., Bandu, M.-T. & Brouty-Boyé, D. (1974) *J. Natl. Cancer Inst.* **52**, 553–559.
10. Tovey, M. G., Begon-Lours, J. & Gresser, I. (1974) *Proc. Soc. Exp. Biol. Med.* **146**, 809–815.
11. Navarro, S., Dion, M., Vodjdani, G., Berlot-Picard, F. & Doly, J. (1989) *J. Gen. Virol.* **70**, 1381–1389.
12. Coulombel, C., Vodjdani, G. & Doly, J. (1991) *Gene* **104**, 187–195.
13. Graham, F. I. & Van der Eb, A. J. (1973) *Virology* **52**, 456–467.
14. Chu, G., Hayakawa, H. & Berg, P. (1987) *Nucleic Acids Res.* **15**, 1311–1326.
15. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
16. Southern, P. J. & Berg, P. (1982) *J. Mol. Appl. Genet.* **1**, 327–341.
17. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **167**, 156–159.
18. Lutfalla, G., Gardiner, K., Proudhon, D., Vielh, E. & Uzé, G. (1992) *J. Biol. Chem.* **267**, 2802–2809.
19. Howley, P. M., Israel, M. A., Law, M.-F. & Martin, M. A. (1979) *J. Biol. Chem.* **254**, 4876–4883.
20. Church, G. M. & Gilbert, W. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1991–1995.
21. Higgins, D. G. & Sharp, P. M. (1988) *Gene* **73**, 237–244.
22. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157**, 105–132.
23. Gaboriaud, C., Uzé, G., Lutfalla, G. & Mogensen, K. E. (1990) *FEBS Lett.* **269**, 1–3.
24. Bazan, J. F. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6934–6938.
25. Thoreau, E., Petridou, B., Kelly, P. A. & Mornon, J.-P. (1991) *FEBS Lett.* **282**, 26–31.
26. Aguet, M. & Blanchard, B. (1981) *Virology* **115**, 249–261.
27. Vanden Broeke, C. & Tovey, M. G. (1991) *J. Interferon Res.* **11**, 91–103.
28. Uzé, G., Lutfalla, G., Eid, P., Maury, C., Bandu, M.-T., Gresser, I. & Mogensen, K. E. (1991) *Eur. J. Immunol.* **21**, 447–451.
29. Nicola, N. A. & Metcalf, D. (1991) *Cell* **67**, 1–4.