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Genetics

Human chromosome 7 carries the β_2 interferon gene

(somatic cell genetics/genomic DNA blot-hybridization/restriction fragment length polymorphism)

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ABSTRACT **A cDNA clone (pAE20-4) corresponding to the 1.3-kilobase human β_2 interferon mRNA was used as a probe in blot-hybridization experiments of DNA from a panel of human–rodent somatic cell hybrids containing overlapping subsets of human chromosomes. The DNA hybridization experiments showed that the human β_2 interferon gene is located on human chromosome 7. This assignment is consistent with previous experimental data in which the expression of the translationally active 1.3-kilobase β_2 interferon mRNA was assayed in various somatic cell hybrids. Blot-hybridization experiments using DNA from different human cell strains and cell lines reveal distinct *Eco*RI restriction fragment length polymorphisms of the human β_2 interferon gene.**

Interferons are vertebrate proteins that render cells resistant to infection by different viruses as a result of inducing cellular metabolic processes that involve synthesis of both RNA and protein (1). Human interferons are further classified as α, β, or γ based on neutralization by appropriate type-specific antisera (1, 2). Studies of the production of biologically active human β interferon (HuIFN-β) and the expression of translationally active (in the *Xenopus laevis* oocyte assay) HuIFN-β mRNA species in cultures of human–rodent somatic cell hybrids exposed to appropriate inducers have indicated that functional HuIFN-β genes are located on different human chromosomes (3–7).

The well-characterized HuIFN-β_1 is the translation product of a 0.9-kilobase (kb) mRNA derived from the intron-free HuIFN-β_1 gene located on the short arm of human chromosome 9 (8). HuIFN-β_2 is the translation product of a 1.3-kb mRNA derived from the intron-containing HuIFN-β_2 gene located on a chromosome other than 9 (7, 9–12). The 1.3-kb HuIFN-β_2 mRNA does not cross-hybridize HuIFN-β_1 cDNA probes in RNA gel-blot hybridization experiments, and vice versa (9, 10). Nevertheless, several different polyclonal antibodies raised against recombinant HuIFN-β_1 or recombinant HuIFN-β_2 neutralized the biological activity of the heterologous protein, and neutralizing monoclonal antibodies raised against recombinant or natural HuIFN-β_1 also cross-neutralized recombinant HuIFN-β_2 (11, 12).

We have used DNA blot-hybridization procedures with a HuIFN-β_2 cDNA probe (10–12) to assign the HuIFN-β_2 gene to human chromosome 7. Furthermore, we have also obtained evidence for restriction fragment length polymorphism of the HuIFN-β_2 gene.

MATERIALS AND METHODS

A HuIFN-β_2 cDNA clone (pAE20-4) that contains an 880-nucleotide cDNA insert in tandem with the simian virus 40 early promoter inserted into the *Hin*dIII site of pBR322 was used as the hybridization probe for the HuIFN-β_2 gene (10–12). Human DNA was obtained from human placenta, diploid fibroblast cell strains (FS-4, GM17, GM258, GM589, GM637B, and GM2767; the GM cell strains were obtained from the Human Genetic Mutant Cell Repository, Camden, NJ), Namalwa lymphoblastoid cell line, and the HeLa cell line, while mouse DNA was obtained from the A9 cell line and hamster DNA, from the E36 cell line. DNA was also obtained from a set of 18 human–mouse and human–hamster somatic cell hybrids that have been described in detail elsewhere (13–17). The human-chromosome composition of these hybrids was determined using standard procedures (14, 17, 18). The procedures used for *Eco*RI digestion of genomic DNA, electrophoresis through 0.8% agarose gels, blotting onto nitrocellulose paper (BA85, Schleicher & Schuell), and hybridization have been described by Sagar *et al.* (13). Briefly, hybridization was in 6× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.5) and 10% (wt/vol) dextran sulfate (Pharmacia) at 65°C for 20–24 hr. The hybridized blots were washed in 2× SSC at room temperature for 30 min and then in 0.3× SSC at 65°C for 1.25 hr. The DNA probe in pBR322 was labeled with [α-^{32}P]dCTP (New England Nuclear) by nick-translation (New England Nuclear kit). Autoradiography usually required an overnight exposure of Kodak XAR-5 film at −70°C with intensifying screens.

RESULTS

HuIFN-β_2 cDNA probes have been used to isolate two distinct genomic DNA clones from a human DNA library in λ phage Charon 4A made from DNA extracted from circulating blood cells of an adult with thalassemia (11, 12). Both clones when transfected into rodent cells lead to the production of biologically active recombinant HuIFN-β_2 (11, 12). One of these (designated IFA-2) contains three *Eco*RI-digested DNA fragments of lengths 6.5, 3.5, and 2.1 kb that hybridize the 880-nucleotide cDNA probe pAE20-4. The other (designated IFA-11) contains *Eco*RI DNA fragments of lengths 6.0, 0.35, and 10 kb of which the 6.0-kb fragment clearly hybridizes to the cDNA probe. The overall restriction map of the 6.5-kb fragment in IFA-2 is very similar to that of the 6.0-kb fragment of IFA-11, and both fragments appear to contain nucleotide sequences corresponding to the carboxyl terminus of the HuIFN-β_2 protein (11, 12). Human genomic DNA clones that appear to correspond to the IFA-11 clone (containing *Eco*RI fragments of 6.0 and 0.35 kb) have also been isolated using a carboxyl-terminal HuIFN-β_2 oligonucleotide probe from another Charon 4A library prepared from human neonatal DNA (clone λH6) (P.B.S. and L.T.M., unpublished data). Thus, it was expected that the HuIFN-β_2 gene would be polymorphic in blot-hybridization analyses of human genomic DNA from different sources.

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Abbreviations: HuIFN-β, human β interferon; kb, kilobase.

Chromosomal Assignment. Fig. 1 illustrates an *Eco*RI digest of human DNA (from Namalwa lymphoblastoid cells) that clearly reveals the presence of the three 6.5-, 3.5-, and 2.1-kb DNA fragments that correspond to the HuIFN-β_2 gene in genomic clone IFA-2. No other hybridization signals are detectable in this lane. Thus these cells do not contain the HuIFN-β_2 gene of the form contained in genomic clone IFA-11 (*Eco*RI fragment of length 6.0 kb). Analyses of mouse and hamster DNA preparations reveal faint cross-hybridizing signals that can be readily distinguished from signals corresponding to the human gene.

DNA preparations from four human–mouse somatic cell hybrids that had been tested earlier (7) for the expression of the 1.3-kb translationally active (in the *Xenopus oocyte* assay) HuIFN-β_2 mRNA were digested with *Eco*RI and analyzed for the presence of the HuIFN-β_2 gene by blot hybridization (Fig. 1). The hybrid (AIM 15aB1) that was observed to clearly express the 1.3-kb HuIFN-β_2 mRNA in the earlier study (see figure 4B in ref. 7) also contains the HuIFN-β_2 gene as detected by blot hybridization. Although its diphtheria toxin-treated derivative (AIM 15aB1/Dpt) failed to express the 1.3-kb mRNA (see figure 4D in ref. 7), it does contain the HuIFN-β_2 gene. DNA preparations from a total of 18 human–mouse and human–hamster somatic cell hybrids were analyzed for the presence of the HuIFN-β_2 gene in a manner similar to that illustrated in Fig. 1. Table 1 summarizes the human chromosomal composition of the various somatic cell hybrids tested, and Table 2 summarizes a detailed analysis of the concordance between specific human chromosomes and the presence of the HuIFN-β_2 gene. The data show that the HuIFN-β_2 gene is located on human chromosome 7.

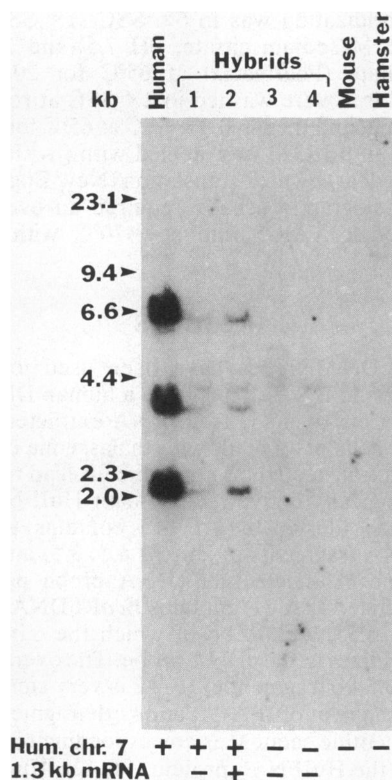

FIG. 1. Blot-hybridization analyses of DNA from human, mouse, hamster, and human–mouse somatic cell hybrids using a HuIFN-β_2 cDNA probe (pAE20-4). DNA samples (20 μg) were digested with *Eco*RI and analyzed by blot hybridization using ^{32}P-labeled pAE20-4. Human DNA was obtained from Namalwa cells, mouse DNA from A9 cells, and hamster DNA from E36 cells. Somatic-cell hybrid preparations were from cell lines AIM 15aB1/Dpt (lane 1), AIM 15aB1 (lane 2), BDA 17b17/Dpt (lane 3), and BDA 17b17/Dpt (lane 4). Expression of the 1.3-kb HuIFN-β_2 mRNA in these hybrids is based on data in ref. 7. The presence (+) or absence (−) of human chromosome 7 (Hum. chr.) and of the 1.3-kb mRNA are indicated.

A confirmation of this assignment is illustrated in Fig. 2. Hybrid 6-5387-21 contains only human chromosome 7 against a mouse background (19). DNA from this hybrid clearly contains the HuIFN-β_2 gene as indicated by the detection of the 6.5-, 3.5-, and 2.1-kb *Eco*RI fragments. In addition, this hybrid contains two intensely hybridizing simian virus 40-specific *Eco*RI fragments that have been described (see figure 2, lane D, in ref. 19).

Restriction Fragment Length Polymorphism of the HuIFN-β_2 Gene. Fig. 2 illustrates that the HuIFN-β_2 gene containing the 6.5-, 3.5-, and 2.1-kb *Eco*RI fragments was detected in DNA preparations from human Namalwa, FS-4, GM258, and GM2767 cells and from a sample of placental DNA. The HuIFN-β_2 gene in the human parent of the AIM (Fig. 1) and the BDA series of hybrids (GM17 and GM589, respectively) also contain these three *Eco*RI fragments (data not shown). Nevertheless, the human–mouse hybrid BDA 14b25 (Fig. 2, lane 10) appears to contain a HuIFN-β_2 gene that may correspond to the gene in genomic clone IFA-11; this gene contains *Eco*RI fragments of length 6.0 and 0.35 kb that clearly hybridize the cDNA probe (Fig. 2). [The restriction map of the human DNA insert in genomic DNA clone IFA-11 contains a 0.35-kb *Eco*RI fragment adjacent to the 6.0-kb fragment on the upstream side (11, 12).] DNA preparations from human HeLa cells contain another form of the gene that includes *Eco*RI restriction fragments of lengths approximately 1.7 and 1.5 kb. Thus the HuIFN-β_2 gene is polymorphic. The detailed structural and functional relationships among the different forms of the HuIFN-β_2 gene remain to be investigated.

DISCUSSION

It is now clear that there exist multiple human β interferon genes. The HuIFN-β_1 gene has been cloned and expressed into biologically active recombinant HuIFN-β_1 by several investigators (reviewed in ref. 20). The HuIFN-β_1 gene was localized to the short arm of chromosome 9 using blot-hybridization procedures near a region that carries the HuIFN-α_1-related gene cluster (9, 21). The HuIFN-β_2 gene (9, 10) has also been cloned and expressed as a biologically active recombinant HuIFN-β_2 (10–12). In the present study we have localized the HuIFN-β_2 gene to chromosome 7 using blot-hybridization procedures and have also observed restriction fragment length polymorphisms of this gene. HuIFN-β-related DNA has also been located on human chromosomes 2 and 4 (13, 22). Although the structural details of these latter loci remain to be investigated, earlier biological data strongly suggest the presence of a functional HuIFN-β gene on human chromosome 2 (3–7).

The assignment of the HuIFN-β_2 gene to chromosome 7 is consistent with previous experimental observations (7). In the previous experiments expression of the 1.3-kb HuIFN-β_2 mRNA was evaluated in poly(I)·poly(C) and cycloheximide-induced human–rodent somatic cell hybrids. The major conclusion drawn from these data was that the results obtained were consistent with the existence of IFN-β genes on different human chromosomes (7). The data then obtained were found to be consistent with the tentative assignment of the HuIFN-β_2 gene to chromosome 5. Nevertheless, it was pointed out that the particular assignments were still tentative and needed to be confirmed by the molecular cloning of the individual IFN-β mRNA species and by the chromosomal mapping of these genes using recombinant DNA procedures. The main reason for this reservation was the possibility that failure to detect expression of the 1.3-kb HuIFN-β_2 mRNA in a hybrid did not necessarily mean the absence of the gene

Table 1. Human chromosomal content of hybrid somatic cell lines assayed by cytogenetic and isozyme analyses

Hybrid cell DNA	Human chromosomes present	Hybridization
BDA 10a3/Dpt	2, 6, 8, 10, 11, 12, 13, 16, 17, 18, 20, X	−
BDA 17b17-1	1, 4, 5, 8, 12, 13, 16, 20, 21, X	−
BDA 10a3	2, 6, 8, 10, 11, 12, 13, 16, 17, 20, X	−
WAV R4dF94a	(6), (10)	−
FRY-4(HLA$^-$,$\beta 2^+$)	1, 3, 4, 10, 11, 13, 14, 18, 21, X	−
FRY-1	4, 9, 11, 13	−
41pt2a	1, 3, 4, 5, 10, 12, 14, 15, 18, 19, 21, X	−
FRY-4(HLA$^+$,$\beta 2^-$)	3, (4), 6, 9, 10, 11, 12, 13, 14, 17, 18, 21, X	−
AHA 3d2-2	(4), (15)	−
AHA 3d2-3	3, 4, 8, 11, 12, 18, 19	−
AHA 3d2-7	4p (missing q12-qter)	−
FRY-4(HLA$^-$,$\beta 2^-$)	3, 4, 6, 11, 13, 14, 21, 22, X	−
6-5387-21	7	+
AIM 15aB1	1, 2, 5, 7, 11, 12, 13, 14, 15, 17, 18, 20, 21, X	+
AIM 15aB1/Dpt	1, 2, 7, 11, 12, 13, 14, 15, 17, 18, 20, 21, X	+
BDA 17b17	1, 2, 3, 4, 5, 6, 9, 12, 13, 15, 16, 18, 20, 21, X	−
BDA 17b17/Dpt	4, 13, 21	−
BDA 14b25	1, 4, 6, 7, 12, 14, 18, X	+

Karyotyping for chromosomes in parentheses was ambiguous primarily because of unclear isozyme data. +, Detection of the HuIFN-β_2 gene using the pAE20-4 cDNA probe; −, failure to detect this gene (see Fig. 1).

in the hybrid cell population. Failure to detect a particular gene product in a hybrid cell population could result from the absence of the gene, the presence of the gene at a low-copy number, or to "gene extinction." Chromosomal mapping using blot-hybridization procedures helps circumvent these problems. Fig. 1 shows that AIM 15aB1 cells that clearly expressed the 1.3-kb HuIFN-β_2 mRNA (see figure 4B in ref. 7) and AIM 15aB1/Dpt cells that did not appear to express the 1.3-kb mRNA (see figure 4D in ref. 7) both contain the HuIFN-β_2 gene. It is noteworthy that human Namalwa cells that contain an intact HuIFN-β_2 gene (Figs. 1 and 2) are unable to express the 1.3-kb HuIFN-β_2 mRNA following the customary poly(I)·poly(C) or viral induction procedures although the 0.9-kb HuIFN-β_1 mRNA is readily expressed in

Table 2. Analysis of concordance between specific human chromosomes and presence of the HuIFN-β_2 gene in the hybrid panel used

Human chromosome	DNA hybridization/chromosome* +/+	+/−	−/+	−/−	Ratio of discordant to total observations
7	4	0	0	14	0.00
2	2	1	3	12	0.22
Y	0	4	0	14	0.22
1	3	1	4	10	0.28
5	1	2	3	12	0.28
14	3	1	4	10	0.28
15	2	2	3	11	0.28
17	2	2	3	10	0.28
19	0	3	2	13	0.28
22	0	4	1	13	0.28
9	0	3	3	12	0.33
20	2	2	4	10	0.33
8	0	3	4	11	0.39
18	3	1	6	8	0.39
6	1	3	5	9	0.44
12	3	1	7	7	0.44
16	0	4	4	10	0.44
21	2	1	7	8	0.44
3	0	4	5	9	0.50
10	0	3	6	9	0.50
11	2	2	7	7	0.50
X	3	1	8	5	0.50
13	2	2	9	5	0.61
4	0	3	11	4	0.78

*Symbols indicate the presence (+/) or absence (−/) of the hybridization signal as related to the presence (/+) or absence (/−) of a particular human chromosome. The number of concordant observations is the sum of the +/+ and −/− observations; the number of discordant observations is the sum of the +/− and −/+ observations.

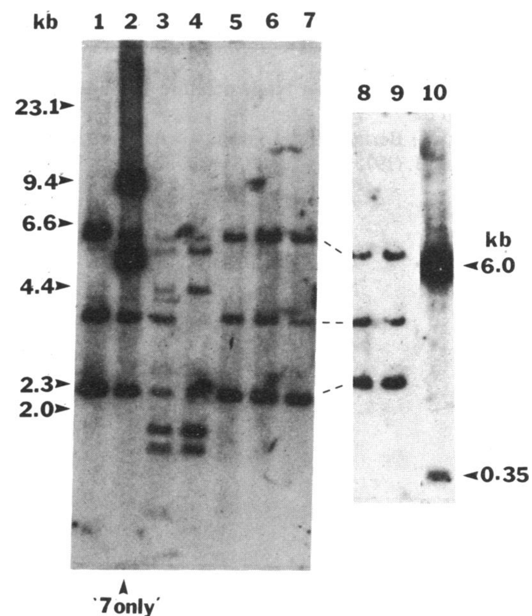

FIG. 2. Blot-hybridization analyses of human DNA from different sources using an HuIFN-β_2 cDNA probe (pAE20-4). DNA samples (10–20 μg) were digested with *Eco*RI and analyzed by blot hybridization using ^{32}P-labeled pAE20-4. Human DNA was obtained from the Namalwa lymphoblastoid cell line (lanes 1 and 8), two different batches of HeLa cells (lanes 3 and 4), the diploid fibroblast cell strain FS-4 (lane 5), the trisomy 21 fibroblast cell strains GM258 (lane 6) and GM2767 (lane 7), and human placenta (lane 9). In addition, DNA from two human–mouse somatic cell hybrids 6-5387-21 (lane 2) and BDA 14b25 (lane 10) was also analyzed. Lane 10 was overexposed to clearly show the 0.35-kb *Eco*RI HuIFN-β_2-specific fragment.

these cells following viral induction (P.B.S. and L.T.M., unpublished data). The presence of a functional HuIFN-β gene other than HuIFN-β_2 on chromosome 5 cannot be excluded (3-6).

The distribution and functional consequences of the various restriction fragment length polymorphisms of the HuIFN-β_2 gene in the human population remain to be explored.

The structure of the HuIFN-β_2 gene is radically different from that of the HuIFN-β_1 gene. While the HuIFN-β_1 gene is a gene without introns, HuIFN-β_2 contains at least three introns (11, 12). Although HuIFN-β_2 shares with HuIFN-β_1 the ability to induce the enzyme 2′-5′ oligo (A) synthetase and class I HLA antigens, the specific activity of HuIFN-β_2 is lower by a factor of 40-100 than that of HuIFN-β_1 when measured in an anti-viral assay (11, 12). Furthermore, there is growing evidence that the biological functions of HuIFN-β_2 are distinct from those of the other interferons. For example, HuIFN-β_2, but not HuIFN-β_1, participates in the mediation of some of the biological effects of "tumor necrosis factor" (23). HuIFN-β_2 appears to be an important autocrine regulator of cell proliferation.

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