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Genetics

Leukocyte and fibroblast interferon genes are located on human chromosome 9

(gene mapping/cell hybrids/cloned genes)

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ABSTRACT **At least eight leukocyte interferon genes (*IFL*) and the single fibroblast interferon gene (*IFF*) have been located on chromosome 9 in humans. In somatic cell hybrids of human and mouse cells containing a normal complement of mouse parental cell chromosomes but reduced numbers of human chromosomes, the human leukocyte and fibroblast interferon DNA sequences were present only when human chromosome 9 was also present.**

The interferons are a family of proteins produced by various animal cells in response to viral infection or other inducing agents such as double-stranded RNA, mitogens, or certain nonviral microorganisms (1–3). Released interferon confers viral resistance on target cells; it also affects cell proliferation and the immune response (1–3).

Three antigenically distinct types of human interferon have been classified according to their cells of origin: fibroblast interferon (FIF or IFN-β), leukocyte interferon (LeIF or IFN-α; produced prominently in β lymphocytes), and immune interferon (IFN-γ; produced by mitogen or antigen-stimulated T lymphocytes) (4–6). Recently fibroblast and leukocyte interferon genes have been cloned by using recombinant DNA techniques (7–13). There may be a single fibroblast interferon gene, but at least eight different leukocyte interferon genes have now been identified (13–15).

Several cytogenetic techniques have been used to localize interferon genes to specific chromosomes. Most approaches involve the use of aneuploid human cells or cross-species hybrid cells (16–20). Chromosomal localization based on interferon production is subject to question because multiple factors may be involved in interferon induction (cf. ref. 2). Cell hybrid studies led several authors to assign fibroblast interferon genes to chromosomes 2 and 5 (18, 19), whereas others have correlated fibroblast interferon production with chromosome 9 (20, 21). The ability of target cells to respond to the antiviral action of interferon has been localized to human chromosome 21 (22). This chromosome probably codes for an interferon receptor molecule (23, 24). Other genetic loci may also be involved in modifying the interferon response. We have been able to assay directly the chromosomal location of the human fibroblast and leukocyte interferon structural genes by hybridization of DNA from human–mouse cell hybrids with radioactive probes from purified cDNA clones of those interferons.

MATERIALS AND METHODS

Parental and Hybrid Cells. Human–mouse cell hybrids derived from chromosomally normal human parental lines were WIL (human WI-38–mouse LTP), REW (WI-38–mouse RAG), and MAR (human GM 654–RAG) (25–27). The other cell hybrids were isolated by using human cell lines with reciprocal chromosome translocations and mouse parental lines. These cell hybrids include TSL (human GM 2808–LM/TK$^-$)which segregates a 17/3 translocation (28), ALR (human AnLy–RAG) which segregates a 9/X rearrangement (29), DUA (human DUV–mouse A9) which involves a X/15 translocation (30), and EXR (human GM 3322–RAG) and XER (human GM 2859–RAG) which segregate translocations involving different breakpoints on chromosomes 11 and X (31). These cell hybrids were constructed and maintained as described (25). Human cell lines preceded by GM were obtained from The Human Genetic Mutant Cell Repository (Camden, NJ). Enzyme markers representing genes assigned to all human chromosomes except the Y chromosome were examined on homogenates of each cell hybrid (32). Chromosome analysis was performed on each cell hybrid as described (33). DNA isolation, isozyme analysis, and chromosome analysis were performed on the same cell passage.

DNA Preparation and Binding of DNA to Filters. High molecular weight DNA was isolated from T-cell lymphoblasts, RAG mouse cells, and human–mouse cell hybrids as described (34, 35). Samples of DNA (10–25 μg) were digested with the restriction endonuclease *Eco*RI (New England BioLabs) with a 10-fold excess of the enzyme over the manufacturer's recommendation for complete digestion. The resulting DNA fragments were separated by electrophoresis through a 1% agarose slab gel and then were transferred to nitrocellulose filters (Schleicher & Schuell) by the method of Southern (36). Fragments of *Hin*dIII-digested λ DNA (New England BioLabs) were used as molecular weight markers.

Hybridization Probes. Fibroblast interferon DNA was isolated from the cDNA clone pFIF3 described by Goeddel *et al.* (10). Two *Pst* I–*Bgl* II fragments corresponding to most of the gene were isolated and labeled with ^{32}P to a specific activity of about 10^8 cpm/μg by calf thymus DNA random fragment-primed synthesis (37). Leukocyte interferon DNA was isolated from cDNA containing fragments of two leukocyte interferon clones designated pLeIF A and D (11, 13). Human growth hormone probe was obtained from the cDNA clone pHGH107 described by Goeddel *et al.* (38).

Hybridization and Washing of Filters. Before hybridization, nitrocellulose filters were prehybridized for 7–12 hr at 42°C in a 10-ml solution containing 50% formamide, 0.75 M NaCl, 75 mM trisodium citrate, 50 mM sodium phosphate buffer

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Abbreviation: kb, kilobase(s).

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(pH 6.5), sonicated denatured salmon sperm DNA (Sigma; 200 μg/ml), and Denhardt's reagent (39) 5 times concentrated. Filters were then hybridized in the same solution to which was added sodium dextran sulfate 500 (Pharmacia) to 10% (40) and ^{32}P-labeled probe (1–10 × 10^6 cpm). Filters were washed in 0.015 M NaCl/0.015 M trisodium citrate/0.1% $NaDodSO_4$ at 60°C unless otherwise noted. Labeled DNA bands were detected by exposing the filters to Kodak x-ray film for 3–7 days in the presence of an intensifying screen (Du Pont Lightning-Plus).

RESULTS

Identification of Leukocyte and Fibroblast Interferon DNA Sequences in DNA Isolated from Human and Human–Mouse Cell Hybrids. DNA from each of human T-cell lymphoblasts, the RAG mouse cell line, and 19 independently derived human–mouse cell hybrids was digested with the restriction endonuclease *Eco*RI, and the resulting DNA fragments were separated by electrophoresis on agarose slab gels. The DNA was then transferred to nitrocellulose filters and hybridized with ^{32}P-labeled interferon probes.

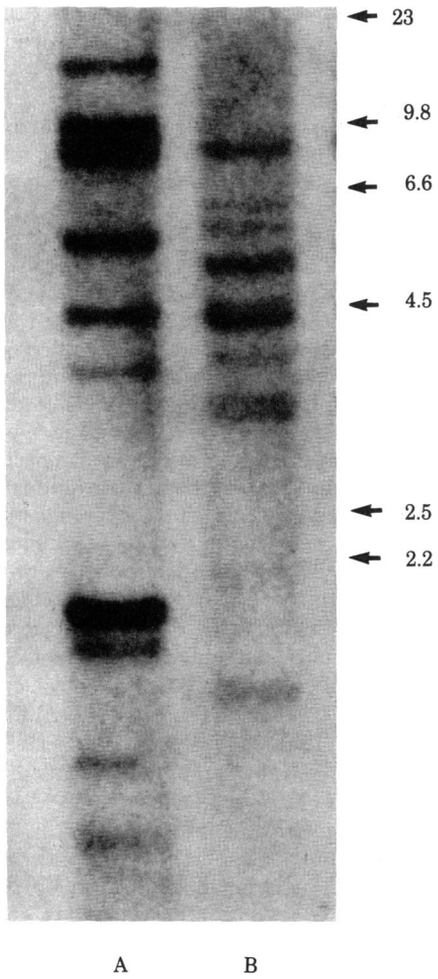

FIG. 1. Leukocyte interferon-related DNA fragments present in *Eco*RI restriction endonuclease digests of human and mouse DNA. Filters were washed in 0.03 M NaCl/0.03 M trisodium citrate/0.1% $NaDodSO_4$ at 50°C. The leukocyte interferon DNA patterns are shown for human T-cell lymphoblasts (lane A) and mouse RAG cells (lane B). Fragments of *Hin*dIII-digested λ DNA were used as molecular weight markers (shown in kb) (New England BioLabs).

The leukocyte interferon probe hybridized with 11 *Eco*RI fragments of 17, 10.0, 9.0, 8.5, 5.3, 4.5, 3.9, 2.0, 1.8, 1.3, and 1.0 kilobase(s) (kb) in the human DNA (Fig. 1, lane A; ref. 13). Eight distinct leukocyte interferon cDNA clones have been isolated previously; two of these clones are cleaved by *Eco*RI (13). The eight cDNA clones share approximately 80–90% DNA sequence homology in the protein coding regions and cross-hybridize under the conditions employed.

In mouse DNA, the leukocyte interferon probe hybridized with nine *Eco*RI fragments of 8.7, 6.5, 6.0, 5.0, 4.4, 4.0, 3.6, 3.5, and 1.5 kb when the filters were washed in 0.03 M NaCl/0.03 M trisodium citrate/0.1% $NaDodSO_4$ at 50°C (Fig. 1, lane B). These sequences were not detected when the filters were washed at a higher stringency 0.015 M NaCl/0.015 M trisodium citrate/0.1% $NaDodSO_4$ (at 60°C) (Fig. 2, lane A) that still detected the human interferon sequences (Fig. 2, lane B). Thus, the mouse *Eco*RI fragments containing interferon DNA sequences differed both in size and degree of homology with the human leukocyte interferon DNA sequences.

Fibroblast interferon cDNA shares only 50% DNA sequence homology with the leukocyte interferon cDNA (9, 11). They do not cross-hybridize under our conditions. Fig. 3, lane A shows the *Eco*RI restriction pattern of human DNA hybridized with fibroblast interferon cDNA probe. This probe hybridized with

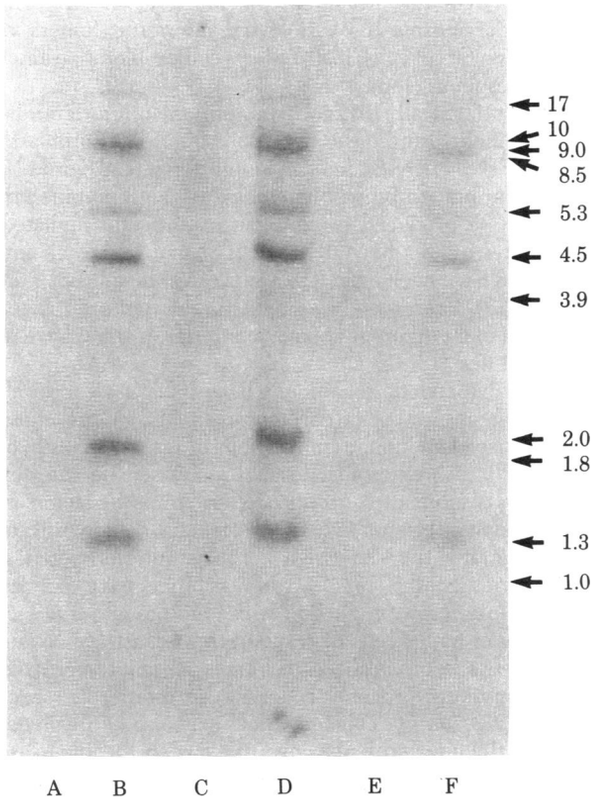

FIG. 2. Human leukocyte interferon-related DNA fragments (shown in kb) present in *Eco*RI restriction endonuclease digests of mouse, human, and human–mouse cell hybrid DNA. Filters were washed in 0.015 M NaCl/0.015 M trisodium citrate/0.1% $NaDodSO_4$ at 60°C. Lanes: A, mouse RAG cells; B, human T-cell lymphoblasts; C, hybrid MAR-2; D, hybrid ALR-2; E, hybrid EXR-9; F, hybrid EXR-5. Lanes B, D, and F contain the human leukocyte interferon DNA sequences. Three restriction fragments of 3.9, 1.8, and 1.0 kb hybridized weakly with the human leukocyte probe. These three DNA fragments were present (*i*) in human DNA and DNA from hybrids ALR-2 and EXR-5 when examined from the original autoradiogram and (*ii*) in other DNA blots not shown.

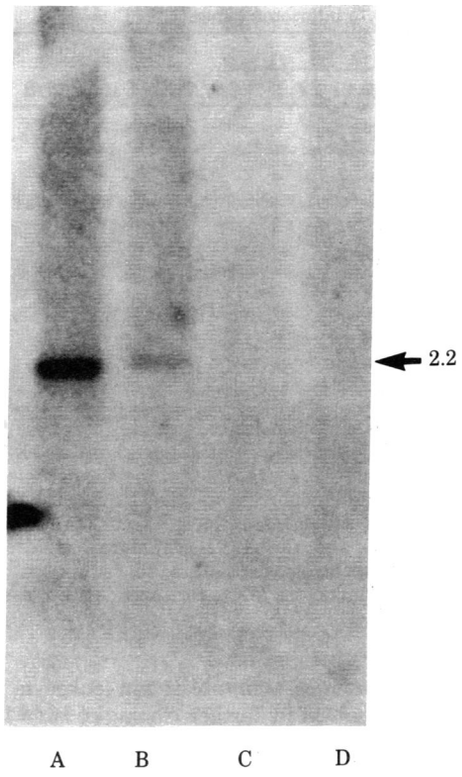

FIG. 3. Fibroblast interferon-related DNA fragments resulting from digestion of human and human–mouse cell hybrid DNA with the restriction endonuclease *Eco*RI. Filters were washed as in Fig 2. Lanes: A, human T-cell lymphoblasts; B, hybrid ALR-2; C, hybrid EXR-9; D, hybrid XER-7. Lanes A and B contain the 2.2-kb human fibroblast interferon-related DNA sequences.

a single *Eco*RI fragment of 2.2 kb. The human fibroblast cDNA did not detect mouse interferon DNA sequences under the conditions used.

DNA isolated from human–mouse cell hybrids was tested for the presence of huma leukocyte and fibroblast interferon DNA sequences. Two out of 19 hybrids analyzed contained human interferon DNA sequences. Both of these hybrids, ALR-2 and EXR-5, contained the full complement of human fibroblast (not shown for EXR-5 in Fig. 3) and leukocyte sequences (see Figs. 2 and 3 for representative data).

The absence of interferon hybridizing sequences in the other 17 hybrids analyzed was not an artifact resulting from poor DNA transfer from the agarose gel or from loss of DNA from the nitrocellulose filter. All of the hybrids contained the mouse interferon sequences, as detected by hybridization with the human leukocyte probe at lower stringency. Further, the human growth hormone cDNA probe hybridized specifically with three *Eco*RI fragments of 9.5, 2.8, and 2.6 kb in DNA associated with human chromosome 17 and a single *Eco*RI fragment of 7.5 kb in mouse DNA evident in all hybrids (34).

Genetic Linkage of Leukocyte and Fibroblast Interferon Genes with Chromosome 9 Enzyme Markers. The chromosome localization of the interferon genes was investigated by examining the segregation patterns of leukocyte and fibroblast hybridizing sequences and enzyme markers previously located on each of the human chromosomes (32). Both the 2.2-kb fibroblast fragment and the 11 leukocyte fragments segregated with the chromosome-9 enzyme markers (41), adenylate kinase-1 (AK1), and aconitase-1 (ACO1) (Table 1). No other enzyme markers correlated with the presence of the interferon sequences. For example, the presence of the chromosome-16 marker adenosine phosphoribosyl transferase (APRT) did not correlate with the presence of interferon sequences in 4 of the 19 cell hybrids; thus, the interferon genes are not located on human chromosome 16. The strict correlation of both fibroblast and leukocyte interferons with the chromosome-9 enzyme markers and lack of correlation with all other enzyme markers indicate that the interferon genes are located on chromosome 9.

Table 1. Segregation of fibroblast interferon and leukocyte interferon with enzyme markers in human–mouse cell hybrids

Chromosome	Enzyme marker	Concordant	Discordant
1	AK2/PEPC	14	4
2	IDH1/MDH1	11	8
3	ACY1	12	7
4	PEPS	11	7
5	HEXB	9	10
6	ME1/SOD2	13	6
7	GUSB	9	8
8	GSR	11	9
9	AK1/ACO1	19	0
10	GOT1	7	10
11	LDHA	8	11
12	LDHB/PEPB	9	9
13	ESD	15	4
14	NP	10	9
15	MPI/PKM2	12	6
16	APRT	15	4
17	GALK	3	16
18	PEPA	8	11
19	GPI	13	6
20	ADA1	7	11
21	SOD1	3	16
22	ACO2	11	7
X	G6PD	4	15

Chromosomal localizations of genes coding for the enzyme markers have been reported (42). The concordant segregation column shows the number of hybrid clones in which the presence of interferon DNA sequences correlated with the presence of a human chromosome marker. The discordant segregation column represents the number of hybrid clones in which these sequences did not correlate with the human chromosome markers. The enzyme markers analyzed by gel electrophoresis were AK2 (adenylate kinase-2), PEPC (peptidase-C), IDH1 (isocitrate dehydrogenase-1), MDH1 (malate dehydrogenase-1), ACY1 (aminoacylase-1), PEPS (peptidase-S), GUSB (β-glucuronidase), GSR (glutathione reductase), AK1 (adenylate kinase-1), ACO1 (aconitase-1), GOT1 (glutamic-oxaloacetic transaminase-1), LDHA (lactate dehydrogenase-A), LDHB (lactate dehydrogenase-B), PEPB (peptidase-B), ESD (esterase-D), NP (nucleoside phosphorylase), MPI (mannose-phosphate isomerase), PKM2 (pyruvate kinase-M2), APRT (adenine phosphoribosyltransferase), GALK (galactokinase), PEPA (peptidase-A), GPI (glucose-phosphate isomerase), ADA1 (adenosine deaminase-1), SOD1 (superoxide dismutase-1), ACO2 (aconitase-2), and G6PD (glucose-6-phosphate dehydrogenase) (32, 42–44).

Assignment of Fibroblast and Leukocyte Interferon Gene Sequences by Chromosome Analysis. Eleven of the 19 cell hybrids were directly analyzed by Giemsa-trypsin staining (33) of their chromosomes. Of these 11 hybrids, only EXR-5 and ALR-2 retained chromosome 9 (Table 2). They also contained the human interferon DNA sequences. Furthermore, EXR-9 was positive for all human chromosomes except 9 and was negative for the interferon sequences (Table 2). These results support the location of the fibroblast and leukocyte interferon genes to chromosome 9 in humans.

ALR-2 retains a normal chromosome 9 and a 9/X translocation (9qter→9p24::Xq12→Xqter) in which the long arm of the X chromosome is translocated to the p terminus of the chro-

Table 2. Human chromosome distribution in cell hybirds segregating fibroblast and leukocyte interferon genes

				Chromosomes																										
Hybrid	*IFL**	*IFF**	*AK1*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	9/X	X/15	15/X	17/3	11/X	X/11
WIL-2	−	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−	−	+	−	+	−	−	−	+	−	+	−						
WIL-8X	−	−	−	−	−	+	+	+	−	+	+	−	+	+	+	−	+	−	−	+	+	+	+	+	−	+	−						
WIL-10S	−	−	−	−	+	+	−	+	−	−	+	−	−	+	−	−	+	−	−	+	+	−	+	+	+	+	−						
TSL-2	−	−	−	−	+	−	−	+	+	−	−	−	+	−	+	−	−	−	−	−	+	−	+	+	−	+	−				+		
TSL-6	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−				+		
ALR-2	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	−	−	−	+					
DUA-5	−	−	−	−	−	+	−	+	−	−	−	−	−	+	−	−	+	+	−	+	+	−	−	+	−	−	−		+	+			
MAR-2	−	−	−	+	+	−	+	−	−	+	−	−	+	−	−	−	−	+	+	+	−	+	+	+	+	+	−						
EXR-5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−					+	+
EXR-9	−	−	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−					+	+
XER-9	−	−	−	−	+	−	+	−	−	−	+	−	+	−	+	−	+	−	−	+	+	−	−	+	−	−	−					+	

In each hybrid, chromosomes were determined by Giemsa-trypsin staining (33). Chromosomes, enzyme markers, and interferon were determined on the same passage. ALR-2 retained both a chromosome 9 and a 9/X translocation (29); DUA-5 retained a chromosome 15 and also X/15 and 15/X translocations (44); TSL-2 and TSL-6 contained a 17/3 translocation (28); EXR-5, EXR-9, and XER-9 retained translocations involving human chromosomes 11 and X. However, EXR and XER hybrids had different human parental cells with different breakpoints on 11 and X. For EXR hybrids, the 11/X translocation is designated (11pter→11q13::Xq22→Xqter) and the X/11 translocation is designated (Xpter→Xq22::11q13→11qter). For XER hybrids, the 11/X translocation is designated (Xqter→Xq13::11p13→11qter) and the X/11 translocation is designated (Xpter→Xq13::11p13→11pter) (31). This terminology follows the Paris system of human chromosome nomenclature (45).

* Human leukocyte interferon genes have been designated *IFL* and the human fibroblast interferon gene *IFF* to correspond with the accepted nomenclature for human genes (42).

mosome-9 short arm (29). The selectable *HPRT* locus (hypoxanthine phosphoribosyl transferase) is encoded on the X chromosome region translocated to chromosome 9p and must be present in ALR hybrids for growth on hypoxanthine/aminopterin/thymidine selection media (46). By counterselecting ALR-2 on 8-azaguanine-supplemented media (cf. ref. 29), a clone, ALR-2 BSAg, was obtained that had lost both the 9/X translocation and the normal chromosome 9. This counter-selected clone was negative for leukocyte and fibroblast interferon DNA sequences and for chromosome 9 enzyme markers. These results further support the localization of the fibroblast and the leukocyte interferon genes to human chromosome 9.

DISCUSSION

The technique of blot hybridization with radioactive probes from purified cDNA clones to filters containing DNA from human–mouse hybrid cells has been utilized to determine the chromosome localizations of a number of human genes, including β globin (47), insulin (35), growth hormone (34), chorionic somatomammotropin (34), prolactin (48), adrenocorticotropin (49), and now the interferon genes. One advantage of this technique is that the structural gene is assayed directly whether or not it is expressed in the cell assayed. Previously, fibroblast interferon genes were localized to human chromosomes 2 and 5 by one group (18, 19) and to human chromosome 9 by another (20, 21). Our results demonstrate that both fibroblast and leukocyte interferon structural genes are located on chromosome 9 in humans.

The chromosome assignment of leukocyte interferon genes has not been reported. Genomic DNA fragments containing closely linked leukocyte interferon genes have been obtained (ref. 14; unpublished data). Thus, the human leukocyte interferon genes appear to be closely linked on chromosome 9. Other gene families having a high degree of sequence homology (greater than 80%) also have been shown to be tightly linked; these include the β globin-like genes on chromosome 11 (47), the α globin-like genes on chromosome 16 (50), and the growth hormone and chorionic somatomammotropin genes on chromosome 17 (34). Human fibroblast interferon DNA sequences contain only 50% sequence homology with the leukocyte interferon sequences (9, 11) but are still located on the same chromosome. The degree of closeness of fibroblast and leukocyte interferon genes may be resolved by further cloning of large DNA fragments. In addition, the localization of these genes on chromosome 9 may be more precisely determined by examining cell hybrids containing different translocated segments of chromosome 9.

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