Somatic Cell and Molecular Genetics, Vol. 14, No. 2, 1988, pp. 123–131

cDNA Cloning and Assignment to Chromosome 21 of *IFI-78K* Gene, the Human Equivalent of Murine *Mx* Gene

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Received 22 June 1987—Final 16 November 1987

Abstract—*Recently we have purified to homogeneity and characterized an interferon-induced human protein (p78 protein) which is the equivalent of the interferon-induced murine Mx protein responsible for a specific antiviral state against influenza virus infection. A cDNA library was constructed using mRNAs from interferon-induced human diploid fibroblasts. cDNA clones coding for the human p78 protein were identified and used to determine the chromosomal location of the corresponding gene (termed* IFI-78K *gene) by hybridization to DNA from a panel of human × rodent somatic cell hybrids. The newly identified gene is located on chromosome 21. This has been confirmed by the observation of a gene dosage effect using chromosome 21 trisomic cells (fibroblasts derived from Down's syndrome patients). Among all interferon-inducible genes mapped so far, the* IFI-78K *gene is the only one located on chromosome 21, together with the gene for the receptor of type I interferon. Our results also provide further evidence for homology between human chromosome 21 and mouse chromosome 16, since the gene encoding the mouse Mx protein (the presumed mouse homolog protein of human p78 protein) has been assigned to chromosome 16.*

INTRODUCTION

The murine interferon (IFN) -regulated Mx protein is responsible for a specific antiviral state against influenza virus infection (1, 2). Since influenza is still a major epidemic disease of man, it was of importance to search for an interferon-regulated protein(s) in human cells equivalent to the murine Mx protein. Recently we have purified to homogeneity and characterized an interferon-induced human protein (p78 protein) that is the equivalent of the murine Mx protein as shown by antigenic relatedness, by induction conditions, by physicochemical properties, and by amino acid analysis (3). The antigenic relationship between the human p78 and the mouse Mx protein has been shown by cross-reaction with the mouse Mx protein of polyclonal antibodies raised against p78 and, conversely, recognition of the p78 protein by polyclonal antibodies raised against the mouse Mx protein (3). Cross-reaction of a monoclonal antibody to Mx protein with a protein of similar molecular weight to the p78 protein in IFN-treated human cells has also been described (4). The p78 protein is mainly induced by type I interferon (IFN-alpha/beta) as is the case for the murine Mx protein (3, 5). The human p78 has a pI of approxi-

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mately 6.3 and an apparent molecular weight of 78,000, similar to the mouse Mx protein (1, 3). The amino acid composition of p78 purified to homogeneity is similar to that of the mouse Mx protein which has already been purified (3, 6). The mouse *Mx* gene is located on chromosome 16 (7). Since there is a syntenic region between murine chromosome 16 and human chromosome 21 (8, 9), a human chromosome involved in the pathogenesis of Down's syndrome and Alzheimer's disease, the chromosomal location of the human interferon-regulated p78 gene is of considerable interest. In the present communication we describe cDNA clones coding for the human p78 protein induced by interferon and show that the location of the corresponding newly identified gene is on chromosome 21.

MATERIALS AND METHODS

Interferons. Recombinant human IFN-αB (>95% pure) was produced in yeast, its final purification involving affinity immunochromatography using monoclonal antibodies. Recombinant human IFN-γ was obtained from Kyowa Hakko (Japan). IFN titers were calibrated against IFN-α (NIH standard G 023-901-527) or against IFN-γ (NIH standard Gg23-901-530).

Labeling of Cells, Cellular Extracts, and Analysis of ^{35}S-Labeled Proteins. Freshly isolated lymphocytes purified by centrifugation on Percol gradients were maintained at 37°C in RPMI supplemented with 5% inactivated horse serum. For labeling, cells were washed with Hanks' balanced salt solution and incubated for 60 min with [^{35}S]methionine (25 μCi/ml) in the same buffer supplemented with 20 mM HEPES, pH 7.4. The labeling was terminated by washing the cells with cold phosphate saline buffer. The cells were then lysed in 1% SDS and the proteins precipitated with ethanol. Analysis of ^{35}S-labeled proteins in the two-dimensional system was performed exactly as described (1).

Construction of cDNA Library. Using the procedure of Gubler and Hoffmann (10) with some modifications (11, 12), a cDNA library was prepared from 2 μg of oligo(dT)-cellulose purified, sucrose formamide gradient-fractionated, cytoplasmic mRNA extracted from human embryonic foreskin diploid fibroblasts (HEF, Flow 7000) treated for 4.5 h with 1000 IU/ml of rIFN-αB.

Hybrid Selected Translation and Immunoprecipitation Procedure. Cytoplasmic RNA was extracted from HEF cells in culture 4 h after the addition of 1000 IU/ml of rIFN-αB. Cytoplasmic RNA from untreated cells served as a control. Total cytoplasmic RNA was sized on a sucrose gradient, and the RNA sedimenting around 23S was used for hybrid selection. Then 100 μg of this RNA was hybridized to 10 μg plasmid DNA immobilized on nitrocellulose filters (3 × 6 mm, Millipore HAWP). We have essentially followed the method described in detail (13) for hybrid selection using nitrocellulose filters. The eluted RNA was translated in rabbit reticulocyte lysate (Amersham International) according to the instructions of the manufacturer. An aliquot of the proteins synthesized in vitro was separated by polyacrylamide gel electrophoresis, and radioactive proteins (from [^{35}S]methionine in the translation system) detected by fluorography. Another aliquot of proteins was immunoprecipitated with monoclonal antibodies specific for the IFN-induced 78-kd protein. The immunoprecipitates were collected on protein A Sepharose and the samples were analyzed on SDS-polyacrylamide gels.

Northern Blot Analysis. The conditions for Northern blot analysis were as described (14). Hybridization was with a B1.1 cDNA derived probe, i.e., a [^{32}P]RNA transcribed from the SP64 construction shown in Figure 2B, as described (15) and used at a concentration of 10^7 cpm/ml.

Somatic Cell Hybrids for Chromosome Mapping. The hybrid cell panel consisted of 16 human × rodent hybrid cell lines selected

as described here. HA hybrids were derived from the fusion of human kidney fibroblasts with mouse cells of the line A9 (HPGRT, i.e., hypoxanthine guanine phosphoribosyltransferase deficient and APRT, i.e., adenine phosphoribosyltransferase deficient). HB hybrids were derived from the fusion of human kidney fibroblasts with mouse cells of the line B82 (TK, i.e., thymidine kinase deficient). HR hybrids were derived from the fusion of human kidney fibroblasts with rat cells of the line Rat2 (TK). HB, HA221, and HR hybrids were selected in hypoxanthine/aminopterin/thymidine medium. The other HA hybrids were selected in azaserine/thymidine medium. JV hybrids were derived from the fusion of human kidney fibroblasts with Jensen sarcoma rat cells JF1 (asparagine synthetase deficient), thus allowing selection in medium lacking asparagine. The medium used to isolate the hybrids was supplemented with ouabain to select against the human parental cells which are more sensitive to this drug than the rodent parental cells. Primary hybrid clones were subcloned (except HA 221); subclones derived from a given primary clone are identified by numbers differing by the last digit only (for instance HB33 and HB34 are derived from the original HB3 hybrid). Hybrids were genetically characterized by chromosomal analysis using standard air-drying techniques, with slight modifications in the fixation procedures (16), and by typing of several IFN-induced genes previously assigned to human chromosomes (17; Marc Wathelet et al., submitted).

Southern Blot Analysis. High molecular weight DNAs isolated from parental and hybrid cells were digested with EcoRI or BamHI (5–10 units/μg of DNA) for 15 h at 37°C and separated on 1% agarose gels. Gels were denatured for 45 min in 0.5 M NaOH, 1.5 M NaCl, neutralized in 1 M Tris HCl, 1.5 M NaCl, pH 7, for 60 min, and soaked in 20× SSC for 20 min. The DNA was transferred either to a nitrocellulose or a nylon filter, which was baked for 2 h at 80°C, prehybridized in 5× SSC, 5× Denhardt, 50% formamide, 0.2 mg/ml salmon sperm DNA, and 1% SDS, and hybridized in the same buffer containing 10^7 cpm/ml of $\alpha[^{32}P]$UTP-labeled cRNA transcribed on either one of the two constructions represented in Figure 2B. After hybridization for 18 h at 50°C, the filter was washed in 2× SSC, 0.1% SDS at 65°C, then in 0.2× SSC, 0.1% SDS at 65°C, and exposed to a Kodak XAR-5 film with intensifying screens at −70°C.

RESULTS

Induction Conditions for p78 mRNA. In freshly isolated lymphocytes the p78 protein was mainly induced by type I IFN (Fig. 1). At approximately 4 h after the addition of IFN, the IFN-induced p78 protein was already a major spot among newly synthesized proteins, indicating the rapid induction of the corresponding mRNA. Cytoplasmic RNA was therefore isolated from human diploid cells at 4.5 h after induction with 1000 IU/ml of human rIFN-αB for the construction of the cDNA library as indicated in Materials and Methods.

Screening and Characterization of cDNA Clones. The determination of a partial sequence at the *N*-terminus of the p78 protein has allowed for the chemical synthesis of a relevant 20-mer oligodeoxynucleotide mixture as a probe for screening cDNA clones. From a library of 30,000 pBR322 recombinants, we selected and purified a single cDNA clone (B1.1), containing a 850-bp insert (Fig. 2B). Three independent pieces of evidence demonstrate unequivocally that the cDNA insert B1.1 belongs to the DNA region coding for the human Mx-related, interferon-induced p78 protein. First, using a hybridization procedure that is independent of base composition of the oligodeoxynucleotide probe (18), we could deduce (by comparison with the dissociation temperature, T_d, of oligodeoxynucleotides of known length) that one of the

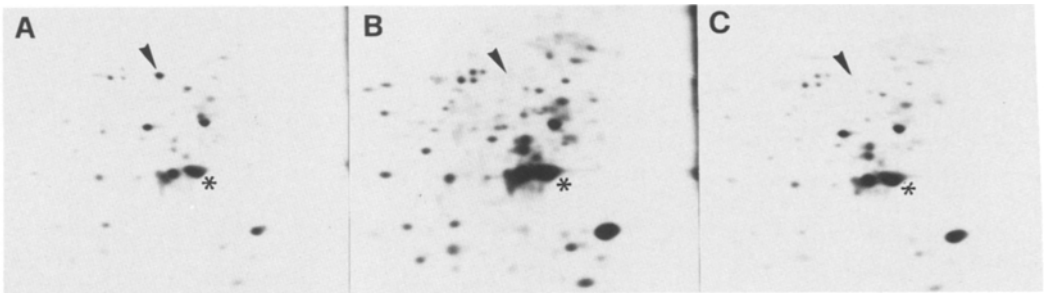

Fig. 1. Induction of the 78-kd protein in human lymphocytes treated with type I or type II interferon. Freshly isolated lymphocytes received 1000 units/ml of human rIFN-αB or of human rIFN-γ. Control lymphocytes received no IFN. Lymphocytes were labeled with [^{35}S]methionine for 60 min starting at 3.5 h after the time of addition of IFN. The radioactive proteins were separated by gel electrophoresis in two dimensions as described (1). The fluorographs represent the radioactive proteins (A) from lymphocytes treated with rIFN-αB, (B) from lymphocytes treated with rIFN-γ, and (C) from control lymphocytes. Arrows indicate the position of 78-kd protein. Asterisks indicate actin.

oligodeoxynucleotide 20-mer probes showed extensive and highly specific homology to the B1.1 cDNA insert. In fact, direct DNA sequencing showed that the B1.1 cDNA insert encodes a polypeptide with the same N-terminus sequence of 10 amino acids as that of the pure p78 (M.A.H. unpublished). Second, in Northern blots of oligo(dT)-cellulose selected RNA from human diploid fibroblasts (HEF), the DNA insert of clone B1.1

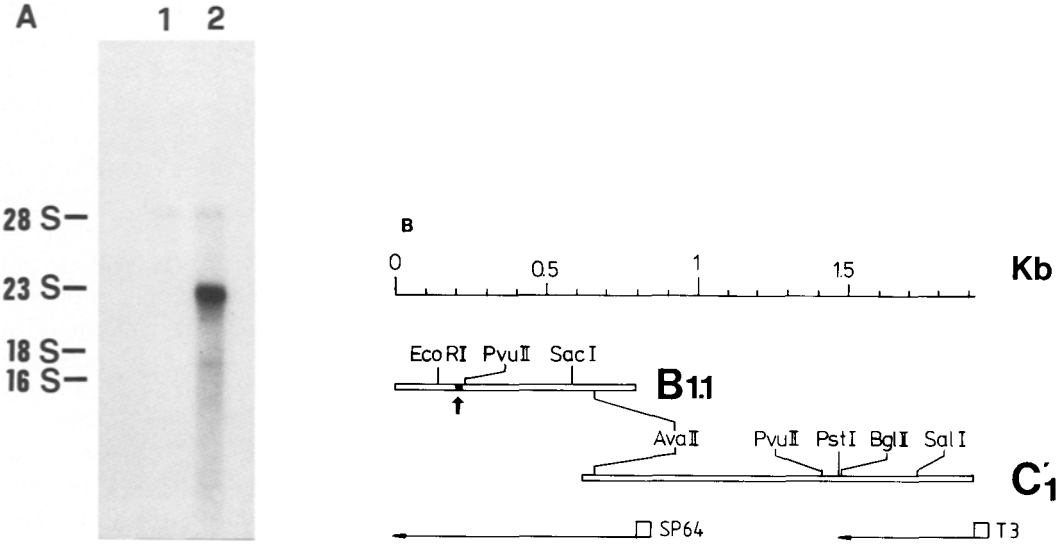

Fig. 2. (A) Northern blot analysis of RNA from interferon-treated or untreated human diploid cells. Hybridization was with a [^{32}P]RNA transcribed from the SP64 construction described in Fig. 2B. Each lane corresponds to 1 μg of cytoplasmic, poly(A)-rich RNA. The weaker invariant signal observed in the 28S region probably corresponds to nonspecific binding of the cDNA probe to residual amounts of 28S rRNA. In fact, a similar nonspecific 28S signal was also observed with a (+)SP64-B.1.1 RNA probe. Lane 1: RNA from untreated cells. Lane 2: RNA from human foreskin fibroblast cells treated with 1000 IU/ml of rIFN-αB for 4.5 h. Rat liver (28S and 18S) and *E. coli* (23S and 16S) rRNAs run in a parallel slot of the same gel and stained with ethidium bromide were used as size markers. (B) Partial restriction map of two p78 cDNA clones. Only the sites that have been determined are indicated. The black square corresponds to the location of the synthetic 20-mer oligodeoxynucleotide used for the screening of our cDNA library. The SP64 construction used for the preparation of a [^{32}P]RNA probe for Northern blot hybridization and the BS-M13+ (Bluescribe^{TM}, Stratagene) construction used for Southern analysis are indicated.

hybridizes to a single RNA of 20–23S corresponding in size to an approx. 3000 nucleotide transcript (an open reading frame of approx. 2100 nucleotides would suffice to encode the p78 protein). This mRNA is not detectable in untreated cells and is strongly induced by the rIFN-αB tratment (Fig. 2A). Finally, using the hybrid selection procedure, the DNA of clone B1.1 has been further characterized to define which specific polypeptides it encodes. The results of Fig. 3 show that the B1.1 DNA selected an IFN-induced mRNA which directed, in a cell-free system, the translation of a polypeptide with the same apparent molecular weight and the same antigenic properties as the p78 protein isolated from IFN-induced Namalwa cells.

The screening of a second pBR322 library of 6500 recombinants by hybridization

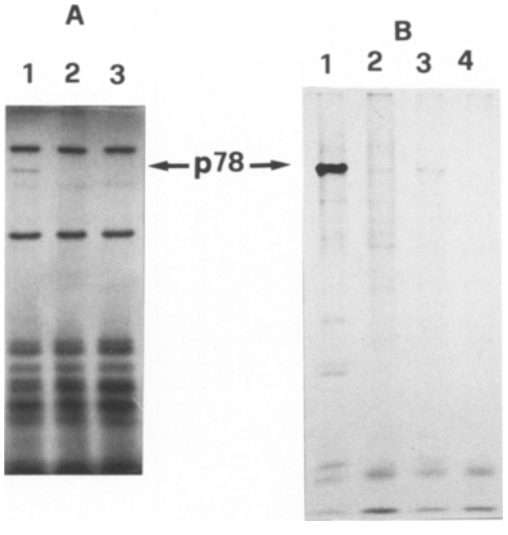

Fig. 3. Characterization of clone B1.1 DNA by hybrid selected translation. ^{35}S-labeled proteins synthesized in reticulocyte lysate using hybrid selected RNA were analyzed on SDS-polyacrylamide. (A) Hybridization using clone B1.1 DNA and interferon-induced RNA (lane 1) or noninduced control RNA (lane 2); hybridization using pBR322 plasmid DNA without insert and interferon-induced RNA (lane 3). (B) Immunoprecipitates of proteins synthesized in the reticulocyte lysate using interferon-induced RNA (lane 1) and control RNA (lane 2) before hybrid selection; immunoprecipitates of proteins translated by IFN-induced mRNA (lane 3) and control mRNA (lane 4) selected by hybridization to B1.1 DNA.

to a B1.1-derived cRNA probe allowed for the selection of a second cDNA clone, C'1, containing a 1300 bp insert overlapping the B1.1 insert by 180 bp (see restriction map, Fig. 2B).

Chromosomal Location of Human IFI-78K Gene. cDNA-derived probes were used to determine the chromosomal location of the gene encoding p78 (termed *IFI-78K* gene to indicate *i*nter*f*eron-*i*nduction) by hybridization to DNA from a panel of human × rodent somatic cell hybrids. The origin of these hybrid clones and their nomenclature are described in Materials and Methods. The human chromosome compositions of the hybrid clones are listed in Table 1. To establish the best conditions for Southern blot analysis of various somatic cell hybrids, EcoRI- and BamHI-digested DNA from mouse and human parental cells were first hybridized with the B1.1 or with the C'1-derived cRNA probes described in Fig. 2B. Figure 4 shows that probe B1.1 revealed one BamHI restriction fragment of 20 kb in human DNA. However, in mouse DNA, a strong smear indicated the presence of mouse repetitive sequence in the B1.1 probe. In contrast, the C'1-derived cRNA probe revealed one 11-kb EcoRI fragment in human DNA and gave no detectable signal in mouse DNA (Fig. 4). We therefore used the C'1-derived cRNA probe to determine the chromosomal location of the human *IFI-78K* gene using EcoRI-digested DNA from a panel of human × rodent somatic cell hybrids. The human 11-kb fragment was present in some of the hybrids, and comparison of the positive and negative hybrid clones were their human chromosome complement allowed the unambiguous assignment of the p78 gene to human chromosome 21 (Table 1).

Since chromosome 21 trisomic cell lines are available (fibroblasts derived from Down's syndrome patients), the assignment of the *IFI-78K* gene to chromosome 21 could be confirmed by the observation of a gene dosage effect. A 50% increase in hybridization of p78

Table 1. Detection of Human *IFI-78K* Gene in Human × Rodent Hybrids[a]

	Human chromosomes																						
Hybrids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
Positive hybrids																								
HA11	−	−	−	+	+	+	−	+	−	−	+	−	−	−	−	−	+	−	−	+	+	−	−	−
HA13	+	−	−	+	+	+	−	+	−	−	+	+	−	−	−	+	+	−	+	+	+	−	+	−
HA14	+	−	+	+	+	+	−	+	−	−	+	+	−	−	−	+	+	−	+	+	+	−	+	−
HA221	−	−	−	+	−	+	+	−	−	−	+	−	−	−	+	−	+	−	+	−	+	−	+	−
HB24	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
HB43	+	+	+	+	−	+	+	+	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	−
HB111	−	+	+	−	−	−	−	+	−	−	+	+	−	+	−	−	+	−	−	−	+	−	−	−
HB112	−	−	+	(−)	−	−	−	(−)	−	−	+	+	−	+	−	−	+	−	−	−	+	−	−	−
HB181	−	+	+	−	−	+	+	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	−	−
HB182	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	−	−
HR40C8	−	−	−	−	−	−	+	−	−	+	−	+	+	+	+	(−)	+	−	+	+	+	+	+	−
HR40C9	−	−	−	−	−	−	+	(−)	(−)	+	−	+	+	+	+	+	+	−	+	−	+	+	+	−
Negative hybrids																								
HB33	−	−	+	+	−	+	−	+	−	−	+	−	−	+	−	−	+	(−)	−	−	−	+	−	−
HB34	−	−	+	−	(−)	(−)	−	+	−	−	−	−	−	+	−	−	+	+	−	−	−	+	−	−
HB142	−	−	−	+	−	−	−	−	−	−	−	−	−	(−)	+	−	+	−	−	−	−	−	−	−
JV211	−	(−)	+	(−)	+	+	+	+	−	+	+	+	−	−	−	+	−	−	−	+	−	+	−	−
Independent discordant clones:	6	5	5	5	7	4	4	4	6	6	3	3	4	3	3	5	2	5	2	3	0	5	3	7

[a] +: chromosome present in at least 20% of the metaphases analyzed. −: chromosome not detected in the metaphases analyzed (at least 10 cells/line) (−): chromosome present in less than 20% of the metaphases analyzed. Independent clones are clones derived from distinct fusion events; nonindependent clones are identified by numbers differing by the last digit only.

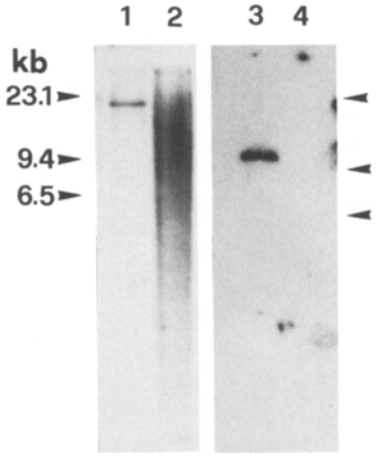

Fig. 4. Southern blot analysis of human and murine genomic DNA, hybridized to two different human p78 cDNA derived probes. Lane 1: BamHI-digested DNA from human kidney fibroblasts. Lane 2: BamHI-digested DNA from murine B82 cells. Lane 3: EcoRI-digested DNA from human kidney fibroblasts. Lane 4: EcoRI-digested DNA from murine B82 cells. Lanes 1 and 2: cRNA probe transcribed on clone B1.1; lanes 3 and 4: cRNA probe transcribed on clone C'1 (see Fig. 2B). The molecular weight markers are HindIII-digested bacteriophage λDNA.

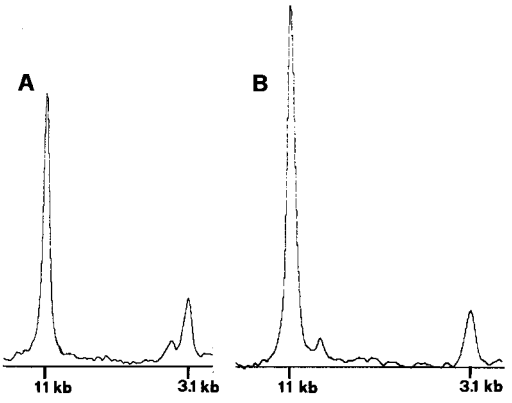

Fig. 5. Densitometric tracings of Southern blots of EcoRI-digested DNA from human diploid cells (A) and from chromosome 21 trisomic cells (B). Hybridization was done simultaneously with the two radioactive probes specific either for the *IFI-78K* gene or for the 2-5 A synthetase gene, as described in the text. The 11-kb fragment is the signal for the *IFI-78K* gene, and the 3.1-kb fragment is the signal for the 2-5 A synthetase gene located on chromosome 12.

cDNA to genomic DNA of chromosome 21 trisomic versus diploid (chromosome 21 disomic) cells should be observed. In order to quantitate this expected difference, we standardized the amount of hybridizing genomic DNA by probing the same Southern blots of EcoRI-digested DNA from trisomic and diploid cells with a DNA probe for the human 2-5A synthetase gene (11) located on chromosome 12 (19). Peak intensity of autoradiograms was recorded by densitometric scanning. The relative intensity of the 11-kb fragment (signal for the *IFI-78K* gene) vs. that of the 3.1-kb fragment (signal for the 2-5 A synthetase gene) was higher in chromosome 21 trisomic than in diploid cells (Fig. 5). The normalized increase in hybridization of the *IFI-78K* gene probe to trisomic cells versus diploid cells varied between 1.24 and 1.6 in two different experiments, whereas the expected value was 1.5.

DISCUSSION

Chromosome 21 is the smallest human chromosome: it carries approximately 1000 genes. Of these, only a few have been identified: RNR4, ribosomal RNA (20, 21); *IFNRA/IFNRB,* interferon receptor (α and β) (22); *SOD-1,* superoxide dismutase, soluble (22); *PRGS,* phosphorybosyl-S-glycinamide synthetase (23); *PAIS,* phosphoribosyl-*S* aminoimidazole synthetase (24); *PFKL,* phosphofructokinase, liver type (25); *CBS,* cystathionine β-synthetase (26); and quite recently the gene encoding the Alzheimer's disease amyloid A4 protein (27–31), the *ETS2* protooncogene (32), and the *ETS2*-related *erg* gene (33). So far four human loci known to be present on human chromosome 21 have been mapped to chromosome 16 in the mouse (8, 9, 34, 35), namely loci *SOD-1, PRGS, IFNRA/IFNRB,* and *ETS2.* Our results provide further evidence for homology between human chromosome 21 and mouse chromosome 16 because the gene encoding the mouse Mx protein (the presumed mouse

homolog protein of human p78 protein), has been assigned to chromosome 16 (7).

We have identified an interferon-induced gene on chromosome 21. Several other interferon-induced genes have already been located on six distinct chromosomes (17; Marc Wathelet et al., in preparation). The *IFI-78K* gene, however, is the only one so far located on chromosome 21, together with the receptor gene for type I IFN (*IFNRA/IFNRB*). It is interesting that the initial mapping of an "antiviral protein" (AVG) to human chromosome 21 (36) later found to correspond to the type I IFN receptor gene (22) might thus still be partially valid. Although p78 and murine Mx protein share a common chromosomal location with the respective *IFNRA/IFNRB* receptor gene, it is unlikely that they are themselves the cell surface receptors since these proteins are not localized at the cell surface (2–4), and murine cells lacking the gene Mx do respond to type I IFN (2). Very recently Jung et al. (37) demonstrated that human chromosome 21 is required for sensitivity to type II interferon (IFN gamma), although the receptor for type II IFN is encoded by chromosome 6. The regulatory element encoded on chromosome 21 seems to be a species-specific transducer that triggers the biological response to type II IFN. These and our own results suggest that chromosome 21 might have a central role in the regulation of the IFN system, and that the colocation of the genes for p78 and for type I IFN receptor on this chromosome might have a biological significance.

The function of the human p78 protein is presently unknown. However, the p78 protein is most likely the human representative of an IFN-induced protein shown to exist in several mammalian species (Michel. A. Horisberger, in preparation). In particular, the homologous IFN-induced mouse Mx protein has been shown to be responsible for a specific antiviral state against influenza virus (1, 2). Evidence suggests that homologous proteins from rat (38) and bovines (39) may also be involved in similar antiviral mechanisms. The homology between p78 and mouse Mx protein is based on similarities with respect to size, pI, amino acid composition, antigenicity, partial amino acid sequence, and induction conditions (3, 6). We therefore speculate that the human p78 protein might also be implicated in antiviral resistance to influenza virus. However, since several results indicate a high degree of conservation of this gene between several animal species (our unpublished results), it is tempting to speculate that the function of the *IFI-78K–Mx* gene family might be more important and broader than initially considered.

We have no evidence to demonstrate that the *IFI-78K* gene plays a role in the pathogenesis of abnormalities associated with chromosome 21. We hope that the cDNA probe to *IFI-78K* gene and the specific monoclonal antibodies to p78 gene product (3) will be valuable tools for investigating pathological situations resulting from trisomy 21. This genetic approach and expression of full-length cDNA clones may also help to define the function of the p78 human protein.

ACKNOWLEDGMENTS

We thank L. DeWit and M. Rivière for excellent technical assistance and H. Hofstetter for support during the initial phase of this work. The work was supported by a grant from the Fund for Joined Basic Research (Belgium) to J.C., by grants from the Fund for Medical Scientific Research (Belgium) to G.H. and to J.C., and by a ULB-Belgian Government grant (Actions de Recherches Concertées) to C.S. Work in Göteborg was supported by the Eric Philip Sörensen Foundation and by the Swedish Cancer Society. C.S. is a Senior Research Associate, G.H. is Research Associate, and M.W. is Research Assistant of the National Fund for Scientific Research (Belgium).

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