cDNA Structures and Regulation of Two Interferon-Induced Human Mx Proteins

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Human cells treated with interferon synthesize two proteins that exhibit high homology to murine Mx1 protein, which has previously been identified as the mediator of interferon-induced cellular resistance of mouse cells against influenza viruses. Using murine Mx1 cDNA as a hybridization probe, we have isolated cDNA clones originating from two distinct human Mx genes, designated MxA and MxB. In human fibroblasts, expression of MxA and MxB is strongly induced by alpha interferon (IFN- α), IFN- β , Newcastle disease virus, and, to a much lesser extent, IFN- γ . MxA and MxB proteins have molecular masses of 76 and 73 kilodaltons, respectively, and their sequences are 63% identical. A comparison of human and mouse Mx proteins revealed that human MxA and mouse Mx2 are the most closely related proteins, showing 77% sequence identity. Near their amino termini, human and mouse Mx proteins contain a block of 53 identical amino acids and additional regions of very high sequence similarity. These conserved sequences are also present in a double-stranded RNA-inducible fish gene, which suggests that they may constitute a functionally important domain of Mx proteins. In contrast to mouse Mx1 protein, which accumulates in the nuclei of IFN-treated mouse cells, the two human Mx proteins both accumulate in the cytoplasm of IFN-treated cells.

Influenza viruses are important human pathogens (35), and one might therefore expect that humans possess a very efficient influenza virus defense system. During the course of viral infections, including influenza virus infections, interferons (IFNs) are synthesized which, in turn, induce an antiviral state in cells surrounding the initial site of virus replication. It is believed that IFNs thus help to limit virus spread and permit the immune system to destroy the invading virus without causing severe tissue damage. The beneficial role of IFNs in host defense against many viruses has been documented in animal model systems (see reference 7 for a review), suggesting that IFN may play a similar role in humans. From an experimental mouse model of influenza virus resistance, we have concluded that IFN exerts its protective effect through the activation of a cellular resistance gene, designated Mx (see reference 30 for a review). Exposure of mouse cells to IFN induces the synthesis of the 72-kilodalton (kDa) Mx1 protein, which, in turn, is capable of selectively blocking the multiplication of influenza viruses (1, 21, 31). The molecular mechanism principally responsible for the IFN-mediated inhibition of influenza virus multiplication is not yet resolved (16, 20, 25).

Evidence in favor of a similar influenza virus defense system in humans includes the observations that IFN can block influenza virus multiplication very efficiently in cultured human fibroblasts (10) and that IFN treatment causes the accumulation of an Mx-related protein in these cells (11, 29). This Mx-related human protein has an apparent molecular mass of about 78 kDa, and, unlike murine Mx1 protein, it accumulates in the cell cytoplasm rather than the nucleus. Although available data are compatible with the view that the Mx-related human protein plays a role in defense against influenza virus, no direct experimental evidence supporting this notion has yet been presented.

One approach to the elucidation of the physiological role of Mx-like proteins of humans is to molecularly clone the human Mx cDNAs and then to express them in transfected cells and to test such cells for newly acquired functions, in particular influenza virus resistance. In this paper we report the first step toward this goal, the isolation and characterization of two Mx-related human cDNAs. We show that the corresponding mRNAs originate from two distinct human Mx genes; the expression of both genes is stimulated by IFN- α , IFN- β , Newcastle disease virus (NDV), and, to a lesser extent, IFN-y. The encoded proteins, designated human MxA and MxB, and Mx-like proteins of other species contain blocks of closely related sequences located near their amino termini. Indirect immunofluorescence analysis with specific antisera indicates that the human MxA and MxB proteins both accumulate in the cytoplasm of IFNtreated cells.

MATERIALS AND METHODS

IFNs. Recombinant human IFN- α_2 (10⁸ U/mg) and recombinant IFN- γ (5 × 10⁷ U/mg) were gifts from Biogen SA, Geneva, Switzerland. Highly purified, natural human IFN- β was purchased from Renschler, Laupheim, Federal Republic of Germany. Confluent cell monolayers were treated with the indicated concentrations of the different IFNs in culture medium containing 2% fetal calf serum. Where indicated, cycloheximide (CHX; final concentration, 75 µg/ml) was added to the culture medium 30 min before the IFN treatment was begun.

Cells. Human fetal lung (HFL) cells and the human glioblastoma cell line T98G (34) were grown in Dulbecco modified minimal essential medium containing 10% fetal calf serum. Confluent monolayer cultures were used for all experiments described here.

NDV. A working stock of NDV was grown in the allantoic

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FIG. 1. Partial restriction maps of MxA and MxB cDNAs. Symbols: \blacksquare , relative positions of the protein-coding regions; \leftarrow , restriction fragments subcloned for subsequent use in S1 hybridization experiments; \leftrightarrow , restriction fragments subcloned into β -galactosidase fusion protein constructs. Fusion proteins were produced in *E. coli* and used to immunize rabbits.

cavity of 10-day-old embryonated eggs. Viral infection was carried out as follows: confluent monolayers of HFL cells in a 90-mm dish were treated for 30 min at 37°C with 8 ml of serum-free medium containing CHX (75 μ g/ml) before 80 μ l of allantoic fluid containing NDV were added. The cell cultures were incubated at 37°C for another 3 h.

Isolation of RNA for cloning experiments and Northern (RNA) blot analysis. Cytoplasmic $poly(A)^+$ RNA was prepared as described previously (28). For some experiments, $poly(A)^+$ RNA was prepared from total cellular RNA isolated by the procedure described by Auffray and Rougeon (2).

Isolation of cDNA clones. Initially, a cDNA library was constructed from poly(A)⁺ RNA of T98G cells treated with IFN- α_2 . cDNA was prepared by the method of Maniatis et al. (17), using the hairpin-S1 method for the synthesis of the second strand. Size-selected cDNA was cloned into the EcoRI site of λ gt10 (14). About 5,000 individual plaques were screened by using the nick-translated PstI-BamHI fragment of the murine Mx cDNA (positions 1181 to 2320 [31]) as a hybridization probe. Two cDNA clones were isolated that differed in their restriction maps but hybridized to the murine Mx cDNA probe. The two cDNA clones were designated MxA and MxB. (MxA corresponds to the previously designated MxC clone, and MxB corresponds to the previously designated MxE clone [M. Aebi, C. E. Samuel, H. Arnheiter, O. Haller, and C. Weissmann, J. Interferon Res. 7:719, 1987].) Additional cDNA libraries were produced in $\lambda gt11$ (36) and λZap (Stratagene Inc., La Jolla, Calif.) from mRNAs of IFN-treated T98G cells and screened with probes specific for MxA (MxA cDNA positions 250 to 1077 in Fig. 4) or MxB (MxB cDNA positions 1690 to 3061 in Fig. 5). Several different, presumably full-length MxA-type cDNA clones were isolated, whereas no long MxB-type cDNA clone was obtained. Therefore, a new cDNA library was prepared in pHG327 (31) by the method of Okayama and Berg (22), with mRNA from HFL cells treated with IFN- α_2 . One presumably full-length MxB-type cDNA clone was isolated from this library by using a fragment of MxB cDNA (positions 185 to 992 in Fig. 5) as the hybridization probe. The nucleotide sequences of representative cDNA clones were determined by the methods of Sanger (27) and Maxam and Gilbert (18).

S1 nuclease protection assays. (i) RNA isolation. Cell monolayers in 90-mm dishes were washed with ice-cold phosphate-buffered saline (PBS) before the RNA was extracted as described by Chomczynski and Sacchi (4), except that cell lysis was performed on the monolayers in culture dish by using 3 ml of their solution D.

(ii) Preparation of S1 probes. The MxA-specific S1 probe was prepared from plasmid pMxA-S1 containing the fragment of MxA cDNA indicated in Fig. 1. pMxA-S1 was constructed as follows. MxA cDNA was first cut with TaqI, and this site was blunted with Klenow polymerase (17). The DNA was next cut with BglII, and the 270-base-pair fragment was recovered from low-melting-temperature agarose. This DNA fragment was then cloned into pHG327 (31) cut with BgIII and PvuII. The MxB-specific S1 probe was prepared from plasmid pMxB-S1 containing the fragment of MxB cDNA indicated in Fig. 1. pMxB-S1 was constructed as follows. MxB cDNA was cut with SacI and Bg/II, and the 275-base-pair fragment was recovered from low meltingtemperature agarose. This DNA fragment was then cloned into pHG327 cut with BgIII and SacI. The β -actin S1 probe was prepared from plasmid pActin-S1, which was con-structed as follows. pHF5 plasmid (8, 23) was cut with Sau3A and BamHI, and the 200-base-pair fragment was recovered from low-melting-temperature agarose. This DNA fragment was then cloned into pHG327 cut with BglII and BamHI, so that the Sau3A and the Bg/II sites became ligated and thus restored the BglII site. To prepare radiolabeled probes, pMxA-S1, pMxB-S1, and pActin-S1 were cut with BglII, treated with alkaline phosphatase, and labeled with 32 P at the 5' end by using T4 polynucleotide kinase (17). The end-labeled DNAs were cut with EcoRI, and the 340-, 670and 570-base-pair fragments, respectively, were recovered.

(iii) Hybridization, digestion with S1 nuclease, and analysis by gel electrophoresis. The assay was performed essentially as described by Berk and Sharp (3). A 15-µg sample of total cellular RNA was transferred to Eppendorf tubes, lyophilized, and dissolved in 20 µl of hybridization buffer, consisting of 40 mM piperazine-N, N'-bis(2-ethanesulfonic acid) (PIPES; pH 6.4), 1 mM EDTA, 400 mM NaCl, 80% formamide, and 0.01 pM of end-labeled S1 probe. A small drop of paraffin oil was added to minimize evaporation during subsequent steps. The samples were incubated for 15 min at 70°C to denature the probe, quickly transferred to a 46°C water bath, and incubated at this temperature overnight. To each tube was then added 250 µl of ice-cold S1 reaction mixture, consisting of 30 mM sodium acetate (pH 4.5), 250 mM NaCl, 1 mM zinc sulfate, 20 µg of carrier DNA per ml, and 200 U of S1 nuclease per ml, and the samples were incubated for 60 min at 37° C. The reaction mixtures were then extracted with phenol, 5 µg of tRNA was added to each sample, and the nucleic acids were precipitated with ethanol. The material was dissolved in 3 µl of 90% formamide gel loading buffer and electrophoresed through a 6% polyacrylamide-8 M urea sequencing gel (18). The gel was exposed to X-ray film at -70° C with an intensifying screen. The radioactivity associated with the gel slices corresponding to bands of interest was quantified by measurement of Cerenkov counts.

Production of antibodies. (i) Fusion protein constructs. A fragment of MxA cDNA containing all sequences downstream from the *Sal*I site was inserted into plasmid pUR291 (24) that was cut with *Sal*I and *Hin*dIII. Ligation at the *Sal*I site joined the β -galactosidase open reading frame (ORF) to that of MxA protein, yielding a fusion protein consisting of β -galactosidase and the fragment of MxA protein indicated in Fig. 1. The *PstI-Hin*dIII fragment of *MxB* cDNA was cloned into pUR291 cut with *PstI* and *Hin*dIII. Ligation at the *PstI* site joined the β -galactosidase ORF to that of MxB protein. Since a fortuitous translational stop codon is located only a few codons downstream of the *Hin*dIII site, the resulting fusion protein consists of β -galactosidase and the fragment of MxB protein indicated in Fig. 1 and only a few additional amino acids.

(ii) Production of fusion proteins. Plasmids were transfected into Escherichia coli BMH 71-18 (24), and ampicillinresistant clones were selected and grown in LB medium at 37°C to mid-log phase. Isopropyl-β-D-thiogalactopyranoside (IPTG; final concentration, 1 mM) was added, and the cultures were incubated for another 4 h before they were harvested and lysed by boiling for 5 min in gel-loading buffer consisting of 125 mM Tris hydrochloride (pH 6.8), 2% sodium dodecyl sulfate (SDS), 10% glycerol, and 5% 2mercaptoethanol. The soluble material was loaded onto 8% polyacrylamide-SDS gels and electrophoresed. The gels were first soaked in ice-cold 250 mM KCl for 5 min and then in ice-cold water for 10 min. The fusion protein was then visible as white band. The gel material containing the fusion protein was cut out, finely minced by being forced through a 21-gauge needle, and diluted with PBS to yield a suspension of about 200 µg of protein per ml.

(iii) Immunizations. Every 2 weeks, female BALB/c mice received intraperitoneal injections of $200-\mu l$ portions of gel material containing MxA or MxB fusion protein. Hyperimmune sera were collected after four immunizations.

Western immunoblot analysis. Protein extracts were prepared by lysing T98G cells in gel-loading buffer containing bromophenol dye. The material was boiled for 3 min, and portions of 100 μ g of protein were electrophoresed through 8% polyacrylamide–SDS gels. After electrophoresis, the proteins were transferred to nitrocellulose by standard procedures. The filters were incubated for 1 h in PBS containing 10% nonfat dry milk and then for 1 h in PBS containing 1% nonfat dry milk and 0.5% mouse hyperimmune serum. The filters were then washed in PBS and incubated for 1 h in PBS containing 1% nonfat dry milk and 0.2% peroxidase-conjugated rabbit anti-mouse immunoglobulin G antibody (Nordic, Tilburg, The Netherlands). The filters were washed in PBS and stained with PBS containing 10% methanol, 300 μ g of 4-chloro-1-naphthol per ml, and 0.01% H₂O₂.

Immunofluorescence analysis. The procedure for immunofluorescence analysis was described by Staeheli et al. (31). Mouse hyperimmune sera were diluted in 1:50 in PBS containing 5% normal goat serum.

RESULTS

Isolation of human Mx-related cDNAs. Southern blot analvses of genomic DNA from several human cell lines indicated that the previously cloned murine Mx cDNA (31) might be a suitable probe for isolating homologous human DNA sequences because low-stringency hybridization experiments demonstrated that several restriction fragments of human DNA contained Mx-related sequences (data not shown). We next tested whether the gene(s) containing these sequences was under IFN control. HFL cells and the glioblastoma cell line T98G were cultured in the presence or absence of 1,000 U of IFN- α_2 per ml for 18 h before RNA extraction. Northern blots from these RNAs were prepared and hybridized with the murine Mx cDNA probe under low-stringency conditions. RNAs of about 2.8 kilobases (kb) were detected in IFN-treated HFL and T98G cells; however, these RNAs were not detected in untreated control cells (data not shown).

To clone these human Mx-related mRNAs, we prepared a cDNA library from mRNAs of T98G cells treated with IFN- α_2 as described in Materials and Methods. The library was screened at low stringency of hybridization with a radiolabeled murine Mx cDNA fragment. Two distinct classes of human cDNA clones, here designated MxA and MxB, were isolated. Using fragments of MxA and MxBcDNAs as hybridization probes to rescreen different cDNA libraries prepared from mRNAs of T98G cells and of HFL cells, both treated with IFN- α_2 , we eventually isolated several MxA and MxB clones containing long inserts (2.5 to 3.0 kb). Partial restriction maps of the longest of these cDNA clones are shown in Fig. 1. The maps of MxA and MxBcDNAs differed substantially from each other, suggesting that the corresponding mRNAs were derived from two distinct Mx-related genes, designated MxA and MxB.

MxA cDNA probes hybridize to an IFN-induced 2.8-kb mRNA, whereas *MxB* probes hybridize to a family of IFNinduced mRNAs. To demonstrate that the cloned *Mx*-related human cDNAs indeed detected IFN-induced mRNAs, we studied the expression of the *MxA* and *MxB* genes in IFN-treated HFL cells. Poly(A)⁺ RNAs isolated from monolayer cultures of HFL cells treated for 5 h either with 1,000 U of IFN- α_2 , IFN- β , or IFN- γ per ml or with IFN-free medium were used to prepare a Northern blot, which was then hybridized sequentially to radiolabeled probes derived from cloned *MxA* and *MxB* cDNAs (Fig. 2).

At high stringency of hybridization, MxA probes hybridized to a single 2.8-kb mRNA from IFN-treated cells. This mRNA, designated MxA mRNA, was abundantly present in HFL cells treated with IFN- α or IFN- β , but was not



FIG. 2. IFN-induced MxA and MxB mRNAs in HFL cells. A Northern blot with poly(A)⁺ RNA (about 1 µg) from HFL cells treated for 5 h with 1,000 U of IFN- α_2 , IFN- β , or IFN- γ per ml was sequentially hybridized to MxA cDNA, MxB cDNA, and human β -actin cDNA. Arrows indicate the gel positions of mRNAs of known sizes, namely, murine MxI mRNA (3.5 kb) and murine Mx2mRNA (2.5 kb).

detectable in untreated control cells. HFL cells treated with IFN- γ also contained *MxA* mRNA, although at a lower concentration. From the apparent size of *MxA* mRNA (2.8 kb), we concluded that our cloned 2.65-kb *MxA* cDNA (Fig. 1; also see Fig. 4) represents a near full-length copy of an *MxA* mRNA.

Radiolabeled MxB cDNA hybridized to a family of IFNinduced mRNAs from HFL cells. The three most abundant of these mRNAs, designated MxB mRNAs, were of 2.8, 3.1 kb, and 4 kb (Fig. 2). These MxB mRNAs were observed in HFL cells treated with IFN- α_2 or IFN- β , but were barely detectable in HFL cells treated with IFN- γ . In IFN-treated HFL cells, MxB RNAs were severalfold less abundant than MxA RNAs. (Note that the Northern blot probed with MxBcDNA in Fig. 2 was exposed about 5 times longer than the Northern blot probed with MxA cDNA.) We detected no MxB mRNAs in untreated HFL cells.

To define the relationships of the different MxB mRNA species, we sequentially reprobed the Northern blot with several small radiolabeled fragments of MxB cDNA. With all fragments except one, we observed efficient hybridization to all three MxB mRNA species. The exception was a probe derived from the 3' untranslated region of MxB cDNA containing sequences downstream of the PstI site located at map position 2.65 kb (Fig. 1). This probe hybridized to the 3.1- and 4-kb MxB mRNAs, but failed to hybridize to the 2.8-kb MxB mRNA (data not shown). Inspection of the MxB cDNA sequence (see Fig. 5) revealed the presence of two copies of the polyadenylation signal sequence AATAAA located about 25 and 300 nucleotides (nt), respectively, from the 3' end of MxB cDNA. The simplest interpretation of these results, therefore, was that our cloned 2.96-kb MxB cDNA (Fig. 1; also see Fig. 5) represents a near-full-length copy of a 3.1-kb MxB mRNA. The 2.8-kb MxB mRNA most probably differs from the 3.1-kb MxB mRNA only by lacking about 300 nt of 3' untranslated sequence owing to polyadenylation of pre-MxB mRNA at the first rather than the second polyadenylation site.

The precise structure of the rare 4-kb MxB RNA is not known. Since this RNA hybridized to all fragments of MxB cDNA tested, even at a very high stringency of hybridization, we believe that the 4-kb MxB transcript represents either unspliced, alternatively spliced, or differentially polyadenylated MxB RNA.

Kinetics of MxA and MxB expression in HFL cells treated with IFN- α_2 . To quantify the MxA and MxB mRNA pools in IFN-treated HFL cells more precisely, we measured MxmRNA concentrations by the S1 nuclease protection technique. Using the restriction fragments indicated in Fig. 1, we established assays as described in Materials and Methods that specifically detected either MxA mRNA or the MxBmRNAs. The high degree of specificity of these S1 assays was demonstrated with in vitro-synthesized MxA and MxBRNAs. Using these two RNAs, we could demonstrate that the S1 probes used were specific for the corresponding mRNAs; no cross-reactivity to the other RNA was observed (data not shown).

In the S1 assay used for MxA mRNA detection (Fig. 3a), the band at 341 nt represents undigested MxA probe, the band at 269 nt is the signal of mature MxA mRNA, whereas the band at 222 nt (asterisk in Fig. 3a) most probably indicates the presence of MxA precursor RNA (see legend to Fig. 3). In the S1 assav used for MxB mRNA detection (Fig. 3b), the band at 671 nt represents undigested MxB probe and the band at 274 nt is the signal of mature MxA mRNA. Because our S1 reaction mixtures further contained a human β-actin probe to provide an internal control to compare the concentrations of RNA in each reaction, our gels showed additional bands which are not of Mx origin. Undigested β -actin probe migrates at 570 nt, whereas β -actin mRNA yields a signal at 175 nt. Our β -actin probe also detected additional RNAs yielding signals of lower molecular masses, designated collectively as Actin RNAs in Fig. 3. These signals presumably result from imperfect hybridization of the β -actin probe to γ -actin mRNA.

We treated HFL monolayer cells for either 1.5, 3, 6, 12, or 24 h with 1,000 U of IFN- α_2 per ml before the RNA was extracted and assayed for the presence of MxA and MxBmRNAs (Fig. 3). Neither MxA nor MxB mRNAs were detectable in untreated control HFL cells, but they accumulated very rapidly after the beginning of IFN treatment and reached maximal concentrations within about 6 h. Thereafter, they decreased slowly. Counting the radioactivity in each signal and comparing these values with those obtained from hybridization of known concentrations of in vitrosynthesized Mx mRNAs, we calculated that HFL cells treated for 6 h with IFN- α_2 contained about 500 molecules of MxA mRNA per cell (Fig. 3a, lane 8) and about 50 molecules of MxB mRNA per cell (Fig. 3b, lane 8). Untreated control cells contained less than 1 MxA or MxB mRNA molecule per cell (Fig. 3, lanes 5 and 11), but after only 90 min of treatment with IFN- α_2 , the levels of Mx mRNA pools were already about 25% of the maximum (Fig. 3, lanes 6). These results demonstrated that IFN- α_2 is a potent inducer of the human Mx genes.

From the Northern blotting experiment described above, we already knew that IFN- β is also capable of inducing human Mx genes very efficiently. The S1 experiment (Fig. 3) demonstrated that this rapid induction of Mx gene expression also occurred when cells blocked with the protein synthesis inhibitor CHX were treated with IFN. HFL cells were treated for 30 min with medium containing 75 µg of CHX per ml before IFN was added. The cells were then kept for 3 h in the medium with CHX and IFN before RNA was



FIG. 3. MxA and MxB mRNA pools in HFL cells under various culture conditions. Monolayer cultures of HFL cells were treated for different times with different IFNs or with NDV in the presence or absence of CHX as indicated. Total RNAs were isolated, and 15 µg of each sample was tested for MxA (A) and MxB (B) mRNA content by S1 analysis. Expected bands are marked. The band at about 220 nt in panel A (asterisk) was not predicted. For two reasons it most probably indicates the presence of unspliced MxA RNAs: (i) this signal is observed at early times of induction only, and (ii) the signal maps to position 1719 of the MxA cDNA, which in the mouse MxI gene marks the end of exon 12 (13). The heavy band at 180 nt is the β-actin signal, and the bands below probably are signals from cross-hybridizing γ -actin RNA. The size marker was pBR322, digested with HaeIII and 5' end labeled with ³²P.

extracted and analyzed. MxA and MxB mRNA pools were slightly higher in HFL cells treated with CHX and IFN than in cells treated with IFN alone (Fig. 3, compare lanes 7 and 18), demonstrating that activation of the Mx genes is not dependent on newly synthesized proteins. Activation of MxA and MxB genes in HFL cells treated with IFN- γ . The Northern blot shown in Fig. 2 revealed that HFL cells treated with IFN- γ contained significant concentrations of both MxA and MxB mRNAs, but because hybridization of that blot with a β -actin probe (Fig. 2) demonstrated that the

lanes contained different amounts of RNA, it was difficult to determine the relative Mx mRNA levels. In a second experiment, we treated HFL monolayer cells with 1,000 U of IFN- γ per ml and determined MxA and MxB mRNA concentrations by S1 analysis at various times after induction. Again, we found MxA and MxB mRNAs in HFL cells treated with IFN- γ . In contrast to cells treated with IFN- α_2 , the pools of MxA and MxB mRNAs constantly increased for at least 24 h after the onset of IFN- γ treatment (Fig. 3; compare lanes 6 to 10 with lanes 12 to 16). After 24 h of IFN treatment, we found about 30 MxA mRNA molecules per cell and about 2 to 3 MxB mRNA molecules per cell. Thus, in HFL cells treated with IFN- γ , maximal MxA and MxBmRNA levels were about 15-fold lower than in cells treated with IFN- α_2 .

Cells in which protein synthesis was blocked with CHX still responded to IFN- γ by activation of Mx genes (Fig. 3, lanes 20), indicating that this activation is a bona fide effect of IFN- γ and is not likely to be an indirect effect of IFN- α , IFN- β , or other inducers that might be synthesized in IFN- γ -treated HFL cells.

Activation of MxA and MxB genes by virus. We tested whether infection of HFL cells with NDV caused an increase in the expression of the human Mx genes. To block the production of virus-induced IFN, we treated HFL cells with CHX before virus infection. At 3 h after infection, about 100 MxA and about 10 MxB mRNA molecules per cell were measured (Fig. 3, lanes 21). Thus, induction by virus of MxA and MxB gene expression does occur in HFL cells, but it is about fivefold less efficient than induction by IFN- α or IFN- β .

Nucleotide sequence of MxA cDNA and deduced amino acid sequence of MxA protein. The sequence of our longest cloned MxA cDNA is shown in Fig. 4. This cDNA contains 2,651 nt of heteropolymeric sequence followed by a poly(A) tail. A long ORF extends from an ATG initiation codon at position 211 to a TAA termination codon at position 2197. The ATG at the beginning of this ORF conforms to the consensus sequences for initiation of translation in vertebrates (15). The encoded protein, designated MxA protein, consists of 662 amino acids with a calculated molecular mass of 75,448 Da, in good agreement with its apparent molecular mass of about 76 kDa estimated by SDS-polyacrylamide gel electrophoresis (see below). MxA protein has an interesting amino acid composition: it contains 13% positively charged residues (5% arginine and 8% lysine) and 16% negatively charged residues (6% aspartic acid and 10% glutamic acid), reminiscent of murine Mx protein. A computer-assisted comparison of the predicted amino acid sequence of MxA protein with published sequences (NBRF-PIR data base, release 17) did not reveal any significant homologies.

Upstream of the beginning of the long ORF there are no additional ATG codons, but, rather, stop codons occur in all three reading frames, indicating that the cloned MxA cDNA contains the complete protein-coding sequence and most of the 5' untranslated region of MxA mRNA. The 3' untranslated region of MxA mRNA consists of about 450 nt and includes the polyadenylation signal sequence AATAAA.

Nucleotide sequence of MxB cDNA and deduced amino acid sequence of MxB protein. The sequence of our longest cloned MxB cDNA (Fig. 5) consists of 2,961 nt of heteropolymeric sequence followed by a poly(A) tail. A long ORF extends from positions 105 to 2249, encoding a putative protein of 715 amino acids with a calculated molecular mass of 81,994 Da. The nucleotide sequence surrounding the first ATG codon of the ORF does not conform to the consensus sequence for initiation of translation in vertebrates (15), suggesting that translation of MxB mRNA might initiate at an ATG codon that is located further downstream. Only the fifth ATG codon of the ORF at position 350 occurs in nucleotide surroundings consistent with a typical start site for translation. Initiation at this alternative position would yield a polypeptide of 633 amino acids, with a calculated molecular mass of 72,445 Da. Western blot analysis (see below) revealed that MxB protein present in IFN-treated T98G cells has an apparent molecular mass of about 73 kDa, rather than the expected 82 kDa if the 5'-proximal ATG codon were used to initiate translation. A computer-assisted comparison of the predicted amino acid sequence of MxB protein with published sequences (PIR data base, release 17) did not reveal any significant homologies.

The 3' untranslated region of MxB mRNA consists of about 700 nt, including two copies of the polyadenylation signal sequence AATAAA. These copies are located 28 and about 300 nt from the poly(A) tail. For the reasons discussed above, we believe that the 2.8- and 3.1-kb MxB mRNAs observed in IFN-treated HFL cells result from alternative polyadenylation at these two sites.

Detection of MxA and MxB proteins with antibodies to β -galactosidase-Mx fusion proteins. We produced β -galactosidase-Mx fusion proteins containing the MxA or MxB protein fragment indicated in Fig. 1 (for details see Materials and Methods) and immunized BALB/c mice with the gelpurified fusion proteins. Hyperimmune sera from these mice were used to stain Western blots prepared from extracts of T98G cells treated with IFN- α_2 (Fig. 6). T98G rather than HFL cells were used for these experiments because large amounts of both MxA and MxB mRNAs were found in IFN- α_2 -treated T98G cells (data not shown); we therefore expected that sufficient levels of the respective Mx proteins might be present within IFN-treated T98G cells to permit detection by Western analysis.

Sera from mice immunized with the MxA fusion protein detected an IFN-induced protein of about 76 kDa (Fig. 6A), roughly corresponding to the MxA protein size predicted from its cDNA sequence. Sera from mice immunized with the MxB fusion protein detected mainly an IFN-induced protein of about 73 kDa (Fig. 6B). A faint signal of a slightly larger protein was also detectable. To demonstrate that the observed signals resulted from specific detection of MxA and MxB proteins by the respective antisera, we stained a Western blot with a mixture of anti-MxA and anti-MxB sera. Two major IFN-induced proteins of similar but distinct migration properties were detectable (Fig. 6C), excluding the formal possibility that both sera detected the same IFNinduced Mx protein. We concluded that the antisera used exhibited a rather high degree of specificity for MxA and MxB proteins, respectively.

The major form of MxB protein synthesized by mouse 3T3 cells transfected with an expression plasmid containing the cloned MxB cDNA migrated on SDS-gels at 73 kDa (data not shown). This result suggested that the cloned MxB cDNA was derived from a representative MxB mRNA molecule and further supported the notion that the fifth ATG codon of the ORF might frequently serve as translation start site.

To determine the intracellular location of MxA and MxB proteins, we used indirect immunofluorescence to analyze T98G and HFL cells before and after treatment for 18 h with IFN- α_2 . Antibodies to MxA protein and antibodies to MxB protein predominantly stained the cytoplasm of IFN-treated cells; no significant nuclear staining was observed (data not shown).

1	GGAATTCTGTGGCCATACTGCGAGGAGATCGGTTCCGGGTCGGAGGCTACAGGAAGACTCCCACTCCCTGAAATCTGGAGTGAAGAACGCCGCCATCCAGCCACCATTCCAAGGAGGTGC
121	AGGAGAACAGCTCTGTGATACCATTTAACTTGTTGACATTACTTTTATTTGAAGGAACGTATATTAGAGCTTACTTTGCAAAGAAGGAAG
241	GCTGATCCAGCTGCTGCAGCCAGCAGAACAACCTGTGCAGCAGATAGCTGGAGAGAGGAGGAGGAGGGAG
361	CCCTGCATCGACCTCATTGACTCCCTGCGGGCTCTAGGTGTGGAGCAGGACCTGGCCCTGCCCGCCATCGCGGACCAGAGCTCCGGGCAAGAGCTCCGTGTTGGAGGCACTG ProCysIleAspLeuIleAspSerLeuArgAlaLeuGlyValGluGInAspLeuAlaLeuProAlaIleAlaValIleGlyAspGInSerSerGlyLysSerSerValLeuGluAlaLeu 60 70 80 90
481	TCAGGAGTTGCCCTTCCCAGAGGCAGCGGGATCGTGACCAGATGCCCGCTGGTGCTGAAACTGAAGAAACTTGTGAACGAAGGATAAGTGAGAGGCGAAGGTCAGTTACCAGGACTACGAG SerGlyValAlaLeuProArgGlySerGlyIleValThrArgCysProLeuValLeuLysLeuLysLeuLysLeuValAsnGluAspLysTrpArgGlyLysValSerTyrGlnAspTyrGlu 100 110 120 130
601	ATTGAGATTTCGGATGCTTCAGAGGTAGAAAAGGAAATTAATAAAGCCCAGAAATGCGCATCGCCGGGGAAGGAA
721	GTCCCGGATCTGACTCTAATAGACCTTCCTGGGCATAACCAGAGTGGCTGTGGGCAATCAGCCTGCTGACATTGGGTATAAGATCAAGACACTCATCAAGAAGTACATCCAGAGGCAGGAG ValProAspLeuThrLeuIleAspLeuProGlyIleThrArgValAlaValGlyAsnGlnProAlaAspIleGlyTyrLysIleLysThrLeuIleLysLysTyrIleGlnArgGlnGlu 170 180 190 210
841	ACAATCAGCCTGGTGGTGGTGGTCCCCCAGTAATGTGGACATTGCCACCACGAGGGCTCTCAGCATGGCCCCAGGAGGTGGACCCCCGAGGGAGACAGGACCATCGGAATCTTGACGAAGCCTGAT ThrIleSerLeuValValValProSerAsnValAsplleAlaThrThrGluAlaLeuSerMetAlaGInGluValAspProGluGlyAspArgThrIleGlyIleLeuThrLysProAsp 220 230 240 250
961	CTGGTGGACAAAGGAACTGAAGACAAGGTTGTGGACGTGGTGCGGAACCTCGTGTTCCACCTGAAGAAGGGTTACATGATTGTCAAGTGCCGGGGCCAGCAGGAGATCCAGGACAAGCGAGACCAGCGG LeuValAspLysGlyThrGluAspLysValValAspValValArgAsnLeuValPheHisLeuLysLysGlyTyrMetIleValLysCysArgGlyGlnGlnGluIleGlnAspGlnLeu 260 270 280 290
1081	AGCCTGTCCGAAGCCCTGCAGAGAGAGAGAGATCTTCTTTGAGAACCACCATATTTCAGGGATCTGCTGGGGGAGGAAGGGAAGGGCACGGTTCCCTGCCTG
1201	CTCATCACCACATATCTGTAAATCTCTGCCCCTGTTAGAAAATCAAATCAAGGAGACTCACCAGAGAATAACAGAGGAGCTACAAAAGTATGGTGTCGACATACCGGAAGACGAAAATGAA LeuIleThrHisIleCysLysSerLeuProLeuLeuGluAsnGlnIleLysGluThrHisGlnArgIleThrGluGluLeuGlnLysTyrGlyValAspIleProGluAspGluAsnGlu 340 350 360 370
1321	AAAATGTTCTTCCTGATAGATAAAATTAATGCCTTTAATCAGGACATCACTGCTCTCATGCAAGGAGAGGAGAGGAAACTGTAGGGGAGGAAGACATTCGGCTGTTTACCAGACTCCGACACGGA LysMetPhePheLeuIleAspLysIleAsnAlaPheAsnGlnAspIleThrAlaLeuMetGlnGlyGluGluThrValGlyGluGluAspIleArgLeuPheThrArgLeuArgHisGlu 380 400 410
1441	TTCCACAAATGGAGTACAATTAATTGAAAACAATTTTCAAGAAGGCCATAAAATTTTGAGTAGAAAAATCCAGAAATTTGAAAAATCAGTATCGGGGAGAAGAGCGGCGCGCGGCGCTTGGGAA PheHisLysTrpSerThrIleIleGluAsnAsnPheGlnGluGlyHisLysIleLeuSerArgLysIleGlnLysPheGluAsnGlnTyrArgGlyArgGluLeuProGlyPheValAsn 420 430 440 450
1561	TACAGGACATTTGAGACAATCGTGAAAACCAGCAAATCAAGGGCACTGGAAGAGCCGGCTGTGGATATGCTACACACCGTGACGGATATGGTCCGGCTTGCTT
1681	AATTTTGAAGAGTTTTTTTAACCTCCACAGAACCGCCAAAGTCCAAAATTGAAGACATTAGAGCAGAACAAGAGAGAG
1801	GTCTACTGCCAGGACCAGGTATACAGGGGTGCATTGCAGAAGGAGGTCAGAAGGAAG
1921	GACTCTTCCATGGAGGAGATCTTTCAGCACCTGATGGCCTATCACCAGGAGGCCAGCAAGCGCATCTCCAGCCACATCCCTTTGATCATCCAGTCTTCATGCTCCAGACGTACGGCCAG AspSerSerMetGluGluIlePheGlnHisLeuMetAlaTyrHisGlnGluAlaSerLysArgIleSerSerHisIleProLeuIleIleGlnPhePheMetLeuGlnThrTyrGlyGln 580 590 600 610
2041	CAGCTTCAGAAGGCCATGCTGCAGGCTCCTGCAGGACAAGGGACAAGGGACACCTACAGCTGGCTG
2161	ACGCAGGCTCGGCCCGGCTTGCCCAGGTTCCCCGGTTAACCACACTCTGTCCAGCCCCGTAGACGTGCACGCAC
2281	TGCTCAGTAGTCAGACTGGATAGTCCGTTCCTGCTTATCCGTTAGCCGTGGTGATTTAGCAGGAAGCCGTGTGAGAGCAGTTTGGTTTCTAGCATGAAGAACAGAGCCCCCACCCTCAGATGCA
2401	CATGAGCTGGCGGGATTGAAGGATGCTGTCTTCGTACTGGGAAAGGGATTTTCAGCCCTCAGAATCGCTCCACCTTGCAGCTCTCCCTTCTCTGTATTCCTAGAAACTGACACATGCTG
2521	AACATCACAGCTTATTTCCTCATTTTTATAATGTCCCTTCACAAAACCCAGTGTTTTAGGAGCATGAGTGCCGTGTGTGT
2641	ATTTCTAGCAG (A) n

FIG. 4. Primary structure of MxA mRNA. The heteropolymeric nucleotide sequence of the longest MxA cDNA clone and the deduced amino acid sequence of MxA protein are shown. The polyadenylation signal sequence AATAAA is underlined.

Comparison of human, mouse, and fish Mx proteins. A comparison of all published vertebrate Mx protein sequences is shown in Fig. 7. The predicted sequences of the human MxA and MxB proteins, and those of hypothetical murine Mx2 and fish Mx-like proteins, were aligned for the best fit with mouse Mx protein (Mx1). The similarities among the five sequences are particularly striking in the regions that correspond to the amino-terminal moiety of

1	AAGAGATGATTTCTCCATCCTGAACGTGCA	GCGAGCTTGTCAGGAAGATCGGAGGTG	CCAAGTAGCAGAGAAAGCATCCCCCAGC	TCTGACAGGGAGACAGCACATGTCT
111	AAGGCCCACAAGCCTTGGCCCTACCGGAGGAGAAGTCAAT LysalahisLysProTrpProTyrArgArgArgSerGInp 10	TTTCTTCTCGAAAATACCTGAAAAAAG heSerSerArgLysTyrLeuLysLysG 20	AAATGAATTCCTTCCAGCAACAGCCACC luMetAsnSerPheGlnGlnGlnProPr 30	GCCATTCGGCACAGTGCCACCACAA OProPheGlyThrValProProGln 40
231	ATGATGTTTCCTCCAAACTGGCAGGGGGCAGAGAAGGAGG MetMetPheProProAsnTrpGInGlyAlaGluLysAspa 50	CTGCTTTCCTCGCCAAGGACTTCAACT LaAlaPheLeuAlaLysAspPheAsnP 60	TTCTCACTTTGAACAATCAGCCACCACC heLeuThrLeuAsnAsnG1nProProPr 70	AGGAAACAGGAGCCAACCAAGGGCA oGlyAsnArgSerGlnProArgAla) 80
351	ATGGGGCCCGAGAACAACCTGTACAGCCAGTACGAGCAGA MetGlyProGluAsnAsnLeuTyrSerGlnTyrGluGlnL 90	AGGTGCGCCCCTGCATTGACCTCATCG ysValArgProCysIleAspLeuIleA 100	ACTCCCTGCGGGGCTCTGGGTGTGGAGCA spSerLeuArgAlaLeuGlyValGluGl 110	GGACCTGGCCCTGCCAGCCATCGCC nAspLeuAlaLeuProAlaIleAla 120
471	GTCATCGGGGACCAGAGCTCGGGCAAGAGCTCTGTGCTGG ValileGlyAspGlnSerSerClyLysSerSerValLeuG 130	AGGCACTGTCAGGAGTCGCGCTTCCCA luAlaLeuSerGlyValAlaLeuProA 140	GAGGCAGCGGAATCGTAACCAGGTGTCC rgGlySerGlyIleValThrArgCysPr 150	GCTGGTGCTGAAAACTGAAAAAGCAG oLeuValLeuLysLeuLysLysGln 160
591	CCCTGTGAGGCATGGGCCGGAAGGATCAGCTACCGGAACA ProCysGluAlaTrpAlaGlyArgIleSerTyrArgAsnT 170	CCGAGCTAGAGCTTCAGGACCCTGGCC hrGluLeuGluLeuGlnAspProGlyG 180	AGGTGGAGAAAGAGATACACAAAGCCCA InValGluLysGluIleHisLysAlaGJ 190	GAACGTCATGGCCGGGAATGGCCGG nAsnValMetAlaGlyAsnGlyArg 200
711	GGCATCAGCCATGAGCTCATCAGCCTGGAGATCACCTCCC GlyIleSerHisGluLeuIleSerLeuGluIleThrSerP 210	CTGAGGTTCCAGACCTGACCATCATTG roGluValProAspLeuThrIleIleA 220	ACCTTCCCGGCATCACCAGGGTGGCTGT spLeuProGlyIleThrArgValAlaVa 230	GGACAACCAGCCCCGAGACATCGGA LASpAsnGlnProArgAspIleGly 240
831	CTGCAGATCAAGGCTCTCATCAAGAAGTACATCCAGAGGG LeuGinilelysAlaleuilelysLysTyrileGinArgG 250	AGCAGACGATCAACTTGGTGGTGGTTC InGlnThrIleAsnLeuValValValP 260	CCTGTAACGTGGACATTGCCACCACGGA roCysAsnValAspIleAlaThrThrGJ 270	GGCGCTGAGCATGGCCCATGAGGTG UAlaLeuSerMetAlaHisGluVal 280
951	GACCCGGAAGGGGACAGGACCATCGGTATCCTGACCAAA AspProGluGlyAspArgThrIleGlyIleLeuThrLysP 290	CAGATCTAATGGACAGGGGCACTGAGA roAspLeuMetAspArgGlyThrGluI 300	AAAGCGTCATGAATGTGGTGCGGAACCT ysSerValMetAsnValValArgAsnLe 310	CACGTACCCCCTCAAGAAGGGCTAC wThrTyrProLeuLysLysGlyTyr 320
1071	ATGATTGTGAAGTGCCGGGGCCAGCAGGAGATCACAAACA MetileVallysCysArgGlyGlnGlnGluIleThrAsnA 330	GGCTGAGCTTGGCAGAGGCAACCAAGA IrgLeuSerLeuAlaGluAlaThrLysI 340	AAGAAATTACATTCTTTCAAACACATCC ysGluIleThrPhePheGlnThrHisPr 350	ATATTTCAGAGTTCTCCTGGAGGAG oTyrPheArgValLeuLeuGluGlu 360
1191	GGGTCAGCCACGGTTCCCCGACTGGCAGAAAGACTTACCA GlySerAlaThrValProArgLeuAlaGluArgLeuThr7 370	CTGAACTCATCATGCATATCCAAAAAT hrGluLeuIleMetHisIleGlnLysS 380	CGCTCCCGTTGTTAGAAGGACAAATAAC erleuProleuLeuGluGlyGlnIleAr 390	GGAGAGCCACCAGAAGGCGACCGAG GGluSerHisGlnLysAlaThrGlu 400
1311	GACTTECECETTECEEEEETEACATCCCCAECCAEEA	CCGACAAGATGTTCTTTCTAATTGAGA	AAATCAAGATGTTTAATCAGGACATCGA	AAAGTTAGTAGAAGGAGAAGAAGTT
	GluLeuArgArgCysGlyAlaAspIleProSerGlnGluA 410	llaAspLysMetPhePheLeuIleGluI 420	ysIleLysMetPheAsnGlnAspIleG 430	uLysLeuValGluGlyGluGluVal 440
1431	GluLeuArgArgCysGlyAlaAspTleProSerGlnGluA 410 GTAAGGGAGAATGAGACCCGTTTATACAACAAAATCAGA ValArgGluAsnGluThrArgLeuTyrAsnLysIleArgG 450	IlaAspLysMetPhePheLeuIleGluI 420 AGGATTTTAAAAACTGGGTAGGCATAG IluAspPheLysAsnTrpValGlyIleI 460	ysIleLysMetPheAsnGlnAspIleG 430 TTGCAACTAATACCCAAAAAGTTAAAA euAlaThrAsnThrGlnLysValLysAs 470	LULYSLEUVAlGluGlyGluGluVal 440 MATTATCCACGAAGAGTTGAAAAA SNIleIleHisGluGluValGluLys 480
1431 1551	GluLeuArgArgCysGlyAlaAspTleProSerGlnGluA 410 GTAAGGGAGAATGAGACCCGTTTATACAACAAAATCAGA ValArgGluAsnGluThrArgLeuTyrAsnLysIleArgG 450 TATGAAAAGCAGTATCGAGGCAAGGAGCTTCTGGGATTTG TyrGluLysGlnTyrArgGlyLysGluLeuLeuGlyPhev 490	LIAASpLySMetPhePheLeuIleGluI 420 AGGATTTTAAAAACTGGGTAGGCATAG SluAspPheLySASnTrpValGlyIleI 460 TCAACTACAAGACATTTGAGATCATCG ValAsnTyrLySThrPheGluIleIleV 500	ysIleLysMetPheAsnGlnAspIleG 430 TTGCAACTAATACCCAAAAAGTTAAAA euAlaThrAsnThrGlnLysValLysAs 470 TGCATCAGTACATCCAGCAGCTGGTGGA alHisGlnTyrIleGlnGlnLeuValGJ 510	LULYSLEUVAIGIUGIYGIUGIUVAI 440 ATATTATCCACGAAGAAGTTGAAAAA MILEILEHISGIUGIUVAIGIULYS 480 AGCCCGCCCTTAGCATGCTCCAGAAA UProAlaLeuSerMetLeuGInLys 520
1431 1551 1671	GIULEUArgArgCysGIyAlaAspTleProSerGInGluA 410 GTAAGGGAGAATGAGACCCGTTTATACAACAAAATCAGAG ValArgGluAsnGluThrArgLeuTyrAsnLysIleArgG 450 TATGAAAAGCAGTATCGAGGCAAGGAGCTTCTGGGATTTG TyrGluLysGlnTyrArgGlyLysGluLeuLeuGlyPhev 490 GCCATGGAAATTATCCAGCAAGCTTTCATTAACGTGGCCA AlaMetGluIleIleGlnGlnAlaPheIleAsnValAlaI 530	LIAASPLYSMetPhePheLeuIleGlui 420 CAGGATTTTAAAAACTGGGTAGGCATAG CluAspPheLysAsnTrpValGlyIleI 460 TCCACTACAAGACATTTGAGATCATCG ValAsnTyrLysThrPheGluIleIleV 500 CAAAAACATTTTGGCGAATTTTTCAACC .ysLysHisPheGlyGluPhePheAsnI 540	ysIleLysMetPheAsnGlnAspIleG1 430 TTGCAACTAATACCCAAAAAGTTAAAA euAlaThrAsnThrGlnLysValLysAs 470 TGCATCAGTACATCCAGCAGCTGGTGGJ alHisGlnTyrIleGlnGlnLeuValG1 510 TTAACCAAACTGTTCAGAGCACGATTGJ euAsnGlnThrValGlnSerThrIleG1 550	ULYSLEUVAIGIUGIYGIUGIUVAI 440 UTATTATCCACGAAGAAGTTGAAAAA mIleIleHisGluGluValGluLys 480 GCCCGCCCTTAGCATGCTCCAGAAA UProAlaLeuSerMetLeuGInLys 520 DAGACATAAAAGTGAAACACACACACGCA UASpIleLysValLysHisThrAla 560
1431 1551 1671 1791	GIULEUArgArgCysGIyAlaAspTleProSerGInGluA 410 GTAAGGGAGAATGAGACCCGTTTATACAACAAAATCAGAG ValArgGluAsnGluThrArgLeuTyrAsnLysIleArgG 450 TATGAAAAGCAGTATCGAGGCAAGGAGCTTCTGGGATTTG TyrGluLysGInTyrArgGlyLysGluLeuLeuGlyPhev 490 GCCATGGAAATTATCCAGCAAGCTTCATTAACGTGGCCA AlaMetGluIleIleGInGlnAlaPheIleAsnValAlaI 530 AAGGCAGAAAACATGATCCAACTTCAGTTCAGAATGGAGC LysAlaGluAsnMetIleGInLeuGInPheArgMetGluG 570	LIAASpLysMetPhePheLeuIleGlui 420 GAGGATTTTAAAAACTGGGTAGGCATAG LUASpPheLysAsnTrpValGlyIleI 460 STCAACTACAAGACATTTGAGATCATCC alAsnTyrLysThrPheGluIleIleV 500 AAAAACATTTTGGCGAATTTTTCAACC ysLysHisPheGlyGluPhePheAsnI 540 CAGATGGTTTTTTGTCAAGATCAGATTT SInMetValPheCysGlnAspGlnIle7 580	ysIleLysMetPheAsnGlnAspIleG 430 TTGCAACTAATACCCAAAAAGTTAAAA euAlaThrAsnThrGlnLysValLysAs 470 TGCATCAGTACATCCAGCAGCTGGTGGA alHisGlnTyrIleGlnGlnLeuValG 510 TTAACCAAACTGTTCAGAGCACGATTGZ euAsnGlnThrValGlnSerThrIleG 550 ACAGTGTTGTTCTGAAGAAAGTCCGAGZ yrSerValValLeuLysLysValArgG 590	ULYSLEUVAIGIUGIYGIUGIUVAI 440 UTATTATCCACGAAGAAGTTGAAAAA mIleIleHisGluGluValGluLys 480 GCCCGCCCTTAGCATGCTCCAGAAA UProAlaLeuSerMetLeuGInLys 520 AGACATTAAAAGTGAAACACAACAGCA UASpIleLysValLysHisThrAla 560 AGAGATTTTTAACCCTCTGGGGACG UGluIlePheAsnProLeuGIyThr 600
1431 1551 1671 1791 1911	GIULEUArgArgCysGIyAlaAspTleProSerGInGIUA 410 GTAAGGGAGAATGAGACCCGTTTATACAACAAAATCAGAG ValargGluAsnGluThrArgLeuTyrAsnLysIleArgG 450 TATGAAAAGCAGTATCGAGGCAAGGAGCTTCTGGGATTTG TyrGluLysGInTyrArgGIyLysGluLeuLeuGlyPhev 490 GCCATGGAAATTATCCAGCAAGCTTTCATTAACGTGGCCA AlaMetGluIleIleGInGInAlaPheIleAsnValAlaI 530 AAGGCAGAAAACATGATCCAACTTCAGTTCAGAATGGAGC LysAlaGluAsnMetIleGInLeuGInPheArgMetGluC 570 CCTTCACAGAATATGAAGTTGAACTTCATTATCCCAGTA ProSerGInAsnMetLysLeuAsnSerHisPheProSerA 610	AlaspLysMetPhePheLeuIleGlui 420 AGGATTTTAAAAACTGGGTAGGCATAG SUASpPheLysAsnTrpValGlyIleI 460 STCAACTACAAGACATTTGAGATCATCG /alasnTyrLysThrPheGluIleIleV 500 AAAAACATTTTGGCGAATTTTTCAACC ysLysHisPheGlyGluPhePheAsnI 540 AGATGGTTTTTTGTCAAGATCAGATTT SInMetValPheCysGInAspGInIle7 580 AATGAGTCTTCGGTTTCCTCCTTTACTG SanGluSerSerValSerSerPheThrC 620	ysIleLysMetPheAsnGlnAspIleG 430 TTGCAACTAATACCCAAAAAGTTAAAA euAlaThrAsnThrGlnLysValLysAs 470 TGCATCAGTACATCCAGCAGCTGGTGGA alHisGlnTyrIleGlnGlnLeuValG 510 TTAACCAAACTGTTCAGAGCACGATTGA euAsnGlnThrValGlnSerThrIleG 550 ACAGTGTTGTTCTGAAGAAAGTCCGAGA yrSerValValLeuLysLysValArgG 590 AAATAGGCATCCACCTGAATGCCTACTT luIleGlyIleHisLeuAsnAlaTyrPf 630	ULYSLEUVAIGIUGIYGIUGIUVAI 440 UTATTATCCACGAAGAAGTTGAAAAA INIIeIIeHisGluGluValGluLys 480 GCCCGCCCTTAGCATGCTCCAGAAA UProAlaLeuSerMetLeuGInLys 520 AGACATAAAAGTGAAACACACACACA UASpIIeLysValLySHisThrAla 560 AGAGATTTTTAACCCTCTGGGGACG UGIUIIePheAsnProLeuGIyThr 600 CCTTGGAAACCAGCAAACGTCTCGCC IeLeuGluThrSerLysArgLeuAla 640
1431 1551 1671 1791 1911 2031	GIULEUArgArgCysGIyAlaAspTIeProSerGInGiuA 410 GTAAGGGAGAATGAGACCCGTTTATACAACAAAATCAGAG ValArgGluAsnGluThrArgLeuTyrAsnLysIleArgG 450 TATGAAAAGCAGTATCGAGGCAAGGAGGCTTCTGGGATTTG TyrGluLysGlnTyrArgGlyLysGluLeuLeuGlyPhev 490 GCCATGGAAATTATCCAGCAAGGCTTTCATTAACGTGGGCCA AlaMetGluIleIleGInGInAlaPheIleAsnValAlaI 530 AAGGCAGAAAACATGATCCAACTTCAGTTCAGAATGGAGG LysAlaGluAsnMetIleGInLeuGInPheArgMetGluG 570 CCTTCACAGGAATATGAAGTTGAACTCTCATTTTCCCAGTA ProSerGInAsnMetLysLeuAsnSerHisPheProSerA 610 AACCAGATCCCATTTATAATTCAGTATTTTATGCTCCGAGA AsnGInIleProPheIleIleGInTyrPheMetLeuArgG	ALAAAAACATTTGAGAAAAGCCA AGGATTTTAAAAACTGGGTAGGCATAG SAGGATTTTAAAAACTGGGTAGGCATAG SUASpPheLysAsnTrpValGlyIleI 460 TCAACTACAAGACATTTGAGAATCATCG ValAsnTyrLysThrPheGluIleIleV 500 AAAAACATTTTGGCGAATTTTTCAACG .ysLysHisPheGlyGluPhePheAsnI 540 CAGATGGTTTTTTGTCAAGATCAGATTT SINMetValPheCysGInAspGInIleT 580 AATGAGTCTTCGGTTTCCTCCTTTACTG SINGLUSerSerValSerSerPheThrC 620 GAGAATGGTGACTCCTTGCAGAAAGCCA SUASnGlyAspSerLeuGInLysAlaA 660	ys IleLysMetPheAsnGlnAspIleG1 430 TTGCAACTAATACCCAAAAAGTTAAAA euAlaThrAsnThrGlnLysValLysAs 470 TGCATCAGTACATCCAGCAGCTGGTGGZ alHisGlnTyrIleGlnGlnLeuValG1 510 TTAACCAAACTGTTCAGAGCACGATTGZ euAsnGlnThrValGlnSerThrIleG1 550 ACAGTGTTGTGTTCTGAAGAAAGTCCGAGZ yrSerValValLeuLysLysValArgG1 590 AAATAGGCATCCACCTGAATGCCTACTT luIleGlyIleHisLeuAsnAlaTyrPf 630 TGATGCAGATACTACAGGAAAAAAATCC etMetGlnIleLeuGlnGluLysAsnAr	ULYSLEUVAIGIUGIYGIUGIUVAI 440 TATTATCCACGAAGAAGTTGAAAAA mIleIleHisGluGluValGluLys 480 GCCCGCCCTTAGCATGCTCCAGAAA UProAlaLeuSerMetLeuGInLys 520 AGGACATAAAAGTGAAACACACACAGCA UASpIleLysValLySHisThrAla 560 AGGACATTTTTAACCCTCTGGGGACG UGIUIlePheAsnProLeuGlyThr 600 CTTTGGAAACCAGCAAACGTCTCGCC ieLeuGluThrSerLysArgLeuAla 640 CTATTCCTGGCTGCTTCAAGAGCAG cgTyrSerTrpLeuLeuGInGluGIn 680
1431 1551 1671 1791 1911 2031 2151	GIULEUArgArgCysGlyAlaAspTleProSerGInGluA 410 GTAAGGGAGAATGAGACCCGTTTATACAACAAAATCAGAG ValArgGluAsnGluThrArgLeuTyrAsnLysIleArgG 450 TATGAAAAGCAGTATCGAGGCAAGGAGCTTCTGGGATTTG TyrGluLysGInTyrArgGlyLysGluLeuLeuGlyPhev 490 GCCATGGAAATTATCCAGCAAGCTTTCATTAACGTGGCCA AlaMetGluIleIleGInGlnAlaPheIleAsnValAlaI 530 AAGGCAGAAAACATGATCCAACTTCAGTTCAGAATGGAGG LysAlaGluAsnMetIleGInLeuGInPheArgMetGluG 570 CCTTCACAGAATATGAAGTTGAACTCTCATTTTCCCAGTA ProSerGInAsnMetLysLeuAsnSerHisPheProSerA 610 AACCAGATCCCATTTATAATTCAGTATTTTATGCTCCCGAG AsnGInIleProPheIleIleGInTyrPheMetLeuArgG 650 AGTGAGACCGCTACCAAGGAGAAGAATCCTTAAGGAGAGAAGAA	A A A A C A C A C A C A C A C A C A C A	ys IleLysMetPheAsnGlnAspIleG 430 TTGCAACTAATACCCAAAAAGTTAAAA euAlaThrAsnThrGlnLysValLysAs 470 TGCATCAGTACATCCAGCAGCTGGTGGT alHisGlnTyrIleGlnGlnLeuValGl 510 TTAACCAAACTGTTCAGAGCACGATTGJ euAsnGlnThrValGlnSerThrIleG 550 ACAGTGTTGTTCTGAAGAAAGTCCGAGT yrSerValValLeuLysLysValArgG 590 AAATAGGCATCCACCTGAAGACAAGTCCTACTT luIleGlyIleHisLeuAsnAlaTyrPf 630 TGATGCAGATACTACAGGAAAAAAATCC etMetGlnIleEuGInGluLysAsnAr 670 CACTCTGTCAATTCTCCAGCAAAGAGAM laLeuCysGlnPheSerSerLysGluIJ 710	ULYSLEUVAIGIUGIYGIUGIUVAI 440 TATTATCCACGAAGAAGTTGAAAAA mIleIleHisGluGluValGluLys 480 GCCCGCCCTTAGCATGCTCCAGAAA UProAlaLeUSerMetLeuGInLys 520 AGGACATAAAAGTGAAACACAACAGCA UASpIleLysValLySHisThrAla 560 AGGACATTTTTAACCCTCTGGGGACG UGIUILePheAsnProLeuGlyThr 600 CCTTGGAAACCAGCAAACGTCTCGCC IeLeuGluThrSerLysArgLeuAla 640 CCTATTCCTGGCTGCTTCAAGAGCAG 197YSerTrpLeuLeuGluGluGlu 680 CCCACTGAAGGGCCGGCGATGCCTGTG eHisEnd
1431 1551 1671 1791 1911 2031 2151 2271	GIULEUArgArgCysGIyAlaAspTIeProSerGInGIUA 410 GTAAGGGAGAATGAGACCCGTTTATACAACAAAATCAGAG ValArgGIuAsnGluThrArgLeuTyrAsnLysIleArgG 450 TATGAAAAGCAGTATCGAGGCAAGGAGCTTCTGGGATTTG TyrGIuLysGInTyrArgGIyLysGIuLeuLeuGlyPhev 490 GCCATGGAAATTATCCAGCAAGGCTTTCATTAACGTGGGCCA AlaMetGIuIIeIleGInGInAlaPheIleAsnValAlaI 530 AAGGCAGAAAACATGATCCAACTTCAGTTAGGAATGGAGC LysAlaGIuAsnMetIleGInLeuGInPheArgMetGIuG 570 CCTTCACAGAATATGAAGTTGAACTCTCATTTTCCCAGTA ProSerGInAsnMetLysLeuAsnSerHisPheProSerA 610 AACCAGATCCCATTTATAATTCAGTATTTATGCTCCGAG AsnGInIIeProPheIleIIeGInTyrPheMetLeuArgG 650 AGTGAGACCGCTACCAAGAGAAGAATCCTTAAGGAGAGAGA	ALAAAAAGTGTGATTTTGGGAAGAAAAGCCA AGGATTTTAAAAACTGGGTAGGCATAG AAGACTACAAGACATTGAGATCATCG ALAAAACATTTGAGACATCATCG ALAAAACATTTGGGGAATTTTTCAACG AJAAAACATTTTGGCGAATTTTTCAACG AJAAAACATTTTGGCGAATTTTTCAACG AJAAAACATTTTGGCGAATTTTTCAACG AJAAAACATTTTGGCGAATTTTTCAACG AJAAAACATTTTGGCGAATTTTTCAACG AJAAAACATTTTGGCGAATTTTTCAACG AJAAAACATTTTGGCGAATTTTTCAACG AJAAAACATTTTGGCGAATTTTCAACG AJAAAACATTTTGGCGAAATTTTCAACG AJAAAACATTTTGGCGAATTTTCAACG AJAAAACATTTTGCGGCGAAAAGTCA AJAAACATTTTGCGGTTCCTCCTTTACG AJAAAACATTTTGCGGTTCCTCCTTTACTG AJAAAACATTTTGGGTCCCTCTGCAGAAAGCCA AJAAACATTTTGCAGGCGCGACACG AJAAAACATTTTGCAGGCGCGCACACG AJAAAACATTTTGCAGGCGCGCACCG AJAAAACATTTGGACTCCTCGGCTCGGCCGCACACG AJAAAACATTTGGAAGCCACTCAGGCGCGCACCGC AJAAAACATTTGGAAGCCCACTCGCTTCGGCGCGCACCGC AJAAAACATTTGGAGTGCCGCTCTGCTTTGGGC	ysIleLysMetPheAsnGlnAspIleG1 430 TTGCAACTAATACCCAAAAAGTTAAAA euAlaThrAsnThrGlnLysValLysAs 470 TGCATCAGTACATCCAGCAGCTGGTGGZ alHisGlnTyrIleGlnGlnLeuValG1 510 TTAACCAAACTGTTCAGAGCACGATTGZ euAsnGlnThrValGlnSerThrIleG1 550 ACAGTGTTGTTGTCTGAAGAAAGTCCGAGZ yrSerValValLeuLysLysValArgG1 590 AAATAGGCATCCACCTGAATGCCTACTT luIleGlyIleHisLeuAsnAlaTyrPf 630 TGATGCAGATACTACAGGAAAAAAATCC etMetGlnIleLeuGlnGluLysAsnAr 670 CACTCTGTCAATTCTCCAGCAAAGAAAAAATCC	ULYSLEUVAIGIUGIYGIUGIVAI 440 TATTATCCACGAAGAAGTTGAAAAA mIleIleHisGluGluValGluLys 480 GCCCGCCCTTAGCATGCTCCAGAAA UProAlaLeuSerMetLeuGInLys 520 AGACATAAAAGTGAAACACACACAGCA UASpIleLysValLySHisThrAla 560 AGAGATTTTTAACCCTCTGGGGACG UGUIIePheAsnProLeuGlyThr 600 CCTTGGAAACCAGCAAACGTCTCGCC teLeuGluThrSerLysArgLeuAla 640 CCTATTCCTGGCTGCTCAGAGAGCAG cgTyrSerTrpLeuLeuGInGluGIn 680 CCCACTGAAGGGCGGCGATGCCTGTG teHisEnd
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FIG. 5. Primary structure of MxB mRNA. The heteropolymeric nucleotide sequence of the longest MxB cDNA clone and the deduced amino acid sequence of MxB protein are shown. The two copies of the polyadenylation signal sequence AATAAA are underlined. The ATG codon proposed to serve as the translation start site is underlined, and the polypeptide sequence preceeding this start codon is shown in parentheses.



FIG. 6. Detection of MxA and MxB proteins on Western blots. T98G cells were grown for 18 h in IFN-free medium (-) or medium containing 1,000 U of IFN- α_2 per ml (+) before total cell lysates were prepared and 100-µg aliquots were loaded into individual lanes of an SDS-8% polyacrylamide gel. Mouse hyperimmune sera were used to stain the Western-blotted Mx proteins. (A) Serum to β -galactosidase-MxA fusion protein; (B) serum to β -galactosidase-MxB fusion protein; (C) 1:1 mixture of the two sera. Relative positions of protein size markers are indicated.

mouse Mx protein, encoded by Mxl exons 3 to 8 (13). In this region, the four mammalian Mx proteins contain a stretch of 53 identical amino acids. Conservation of the sequence of this particular stretch of amino acids is also remarkably high in the fish Mx-like protein (33), with 49 of these 53 amino acids being identical.

To learn more about the relationships of the two mouse and the two human Mx protein sequences, we estimated the MOL. CELL. BIOL.

degrees of sequence similarity by simply calculating the numbers of identical amino acids at corresponding positions, limiting the analysis to the 631 amino acids of mouse Mx1 protein (Fig. 7). By this analysis, the human MxA and mouse Mx2 protein are the most closely related, showing 77% sequence identity (Fig. 8), whereas the two human proteins are only 63% identical. The two mouse proteins are very similar at their N termini, but the overall identity of these two proteins is only 73%. Human MxB and mouse Mx1 protein are the least closely related, showing only 56% sequence identity.

DISCUSSION

Using murine MxI cDNA as a hybridization probe, we have identified two classes of human Mx-related cDNAs. Sequence analysis revealed that the corresponding mRNAs must originate from two distinct human Mx genes, which we have designated MxA and MxB. A second Mx gene has recently been identified in the mouse system (32). In bovines, Mx-related proteins are most probably encoded by more than one gene (9), and there is evidence for three Mx-related genes in the rat (19). Thus, small Mx gene families are present in the genomes of all mammals studied to date.

Expression of the two human Mx genes is under tight control by IFN, like expression of the Mx genes of mice (28), rats (19), and cattle (9). In uninduced cells, Mx genes are not expressed at detectable rates; however, cells exposed to IFN- α , IFN- β , or NDV rapidly accumulate as many as a few hundred Mx transcripts per cell. IFN- γ is a less potent inducer of Mx genes in all systems that were studied (6, 19, 30).

MxA protein is most probably identical to the previously

mu Mxl mu Mx2 hu MxA hu MxB fish	Exon 2 <> Exon 3 MDSVNNLCRHYEEKVRPCIDLIDTLRALGVEQDLALPA MVLSTEENTGVDSVNLPSGETGLGEKDQESQS. MVVSEVDIARADPAAASHPLLLNCDATVAQKNPGSVAESQS. MSKAHKPWPYRRRSQFSSRKYLKKEMNSFQQQPPPFGTVPPQMMFPPNWQGAEKDAAFLAKDFNFLTLNNQPPPGNRSQPRA.GPEYSQQS.SK ITM.T.NQQS.S.K.	38 65 72 120
mu Mx1 mu Mx2 hu MxA hu MxB fish	Exon 3 <> Exon 4 Exon 5 IAVIGDQSSGKSSVLEALSGVALPRGSGIVTRCPLVLKLRKLKEGEEWRGKVSYDDIEVELSDP SEVEEAINKGQNF IAGVGLGISDKLISLDVSSPNVPDLTLIDLPGITRVAVGNQPA 	158 185 192 239
Exon mu Mx1 mu Mx2 hu MxA hu MxB fish	5 <> Exon 6 Exon 7 <> DIGRQIKRLIKTYIQKQETINLVVPSNVDIATTEALSMAQEVDPEGDRTIGULTKPDLVDRGAEGKVLDVMRNLVYPLKKGYMIVKCRGQQDIQEQLSLTEAPQKEQVFFKDHSYFSLL Exon 7 <>	278 305 312 359
Ex mu Mx1 mu Mx2 hu MxA hu MxB fish	on 8 Exon 8 <> Exon 9 Exon 9 <> Exon 10 Exon 10 <> Exon 12 Exon 10 <> Exon 12 LEDGKATVPCLAERLTEELTSHICKSLPLLEDQINSSHQSASEELQKYGADIPEDDRTRMSFLVNKISAFNRNIMMLIQAQETVSEGDSRLFTKLRNEFLAMDDHIEEYFKKDSPEVQSK A.IN.KEN.KEMSEKTF.IE.N.QD.TA.V.GE.N.A.ECR.K.S.SKE.KN.E.GYAVLYNE K.S.ITN.KET.RITVENEK.F.ID.N.QD.TA.W.GE.G.I.R.H.HK.STI.NN.QGEHKLING 	1 398 425 432 479
mu Mx1 mu Mx2 hu MxA hu MxB	Exon 11 <> Exon 12 Exon 12 Exon 12 <> Exon 13 MKEFENQYRGRELPGFVDYKAFESIIKKRVKALEESAVNMLRRVTKMVQTAFVKILSNDFGDFLNLCCTAKSKIKEIRLNQEKEAENLIRLHFQMEQIVYCQDQVYKETLKTIREKEAEK VMAKN.T.N.RRQI.T.P.IE.HT.EI.RATSVSEKN.SE.Y.HR.TLEDEMSKII.RGA.QKV.EE IQKN.RT.T.V.QQIP.D.HT.D.RL.TDVSIKN.EE.F.HRED.AE.R.G.KRGA.QKV.L.E VEKY.KK.L.N.T.I.VHQYIQQ.V.P.LS.QKAMEII.Q.INVAKKH.E.F.NQ.VQ.T.ED.KVKHTAKM.Q.Q.RM.FI.SVV.KV.EIFNP	518 545 552 599
mu Mx1 mu Mx2 hu MxA	EXON 13 <> EXON 14 EKTKALINPATFONNSOFPOKGLTTTEMTOHLKAYYOECRNNIGROIPLIIQYFILKTFGEEIEKMMLOLLQDTSKCSWFLEEQSDTREKKKFLKRRLLRLDEAROKLAKFSD .KTKHGTSSSS.SQDLQTSSMA.IFN.R.AHNR.SSHVM.A.RLQ.GKDSL.KS.RE.A.AQ.RRPG .K.KSWDFGA.SS.ATDSSME.IFM.H.ASKR.SSHF.M.Q.Y.QQLQ.AKDT.L.K.RSD.RE.A.TQ.RR.Q.PG .K.KSWDFGA.SS.ATDSSME.IFM.H.ASKR.SSHF.M.PENESIO A MIL FWDWY L.O.F AT DDI F.TV TO HA CO SKETH	631 655 665 716

FIG. 7. Comparison of amino acid sequences of murine Mx protein (mu Mx1 [31]), hypothetical murine Mx2 protein (mu Mx2 [32]), human MxA protein (hu MxA), human MxB protein (hu MxB), and putative fish Mx protein (fish [33]). The sequences are aligned for the best fit relative to murine Mx1 protein (top sequence). Dots indicate the presence of identical amino acids at corresponding positions in murine Mx protein and in the Mx-related protein. The locations of the exon borders in the murine Mx1 gene are indicated.



FIG. 8. Schematic presentation of relationships of mouse and human Mx proteins. The percentages of identical amino acids at corresponding positions are indicated. The calculations are based on the alignment shown in Fig. 7.

described IFN-induced human protein that is recognized by anti-mouse Mx protein monoclonal antibody 2C12 (29). Both proteins have molecular masses of about 76 kDa, and both are accumulated to very high levels in the cytoplasm of HFL cells and T98G cells treated with IFN- α_2 . Furthermore, expression of MxA but not MxB cDNA in transfected mouse cells gives rise to a 2C12 monoclonal antibody-reactive protein (J. Pavlovic, unpublished results). Finally, the Mx protein purified from IFN-treated human foreskin fibroblasts by immunoaffinity chromatography with antibody 2C12 has the same amino-terminal sequence (G. Weitz, J. Bekisz, K. Zoon, and H. Arnheiter, J. Interferon Res., in press) as we predicted for MxA protein.

MxA protein is probably also identical to the IFN-induced p78 (IFI-78K) protein described by Horisberger and Hochkeppel (11). In addition to having similar molecular masses, the two proteins show the same intracellular location and are both induced by IFN- α and to a much lesser extent by IFN- γ (6). Furthermore, the restriction maps of MxA cDNA (Fig. 1) and *IFI-78K* cDNAs (12) are very similar, and the predicted N-terminal sequence of MxA protein is identical to that of p78 (M. Horisberger, personal communication). Horisberger et al. (12) have shown that the *IFI-78K* gene maps to human chromosome 21. We will show elsewhere that MxA and MxB both map to chromosome 21 (P. Huber and O. Haller, unpublished results).

MxB is probably a novel protein. In diploid fibroblasts (Fig. 3) or peripheral blood lymphocytes (data not shown) treated with IFN- α_2 , the steady-state levels of MxB mRNA are 3 to 10 times lower than those of MxA mRNA. MxB protein concentrations in these cells are marginally high enough to permit detection by our anti-MxB sera. The cell line T98G is exceptional in that it responds to IFN- α_2 by synthesizing large amounts of both MxA and MxB mRNAs. In these cells, MxB protein is easily detectable by the Western blotting technique. In IFN-treated T98G cells as well as in mouse cells transfected with appropriate MxBcDNA constructs (J. Pavlovic, unpublished results), MxB protein accumulates in the cell cytoplasm. Thus, both human Mx proteins are confined largely to the cytoplasm. This is in marked contrast to the observations with rodent cells, in which Mx proteins are found in the nuclei of IFN-treated cells (5, 19). We have searched for additional human Mxgenes that might encode a nuclear Mx protein, but have failed to find evidence for them; all cDNA clones with Mx-related sequences that we isolated proved to be derived from either MxA or MxB mRNAs (data not shown).

Western blot analysis revealed that MxB protein present in IFN-treated T98G cells has an apparent molecular mass of about 73 kDa, although the ORF of the cloned MxB mRNA predicts a 715-amino-acid protein with a calculated molecular mass of about 82 kDa. One possible explanation of this difference would be that MxB protein has aberrant migration properties on SDS-polyacrylamide gel electrophoresis. Another possibility would be that the 82-kDa translation product of MxB mRNA is a precursor of the mature 73-kDa MxB protein. We favor a third possibility, that translation of MxBmRNA does not initiate at the first ATG codon of the long ORF but rather at the fifth ATG codon, yielding a protein of about 72.5 kDa. Indeed, the -3/+4 flanking nucleotide context of the ATG codon at the beginning of the ORF is not optimal for translation initiation (15): CACATGT is considered to be a very poor initiation signal. By contrast, the fifth ATG codon of the ORF has a favorable -3/+4 flanking nucleotide context, GCAATGG. Initiation of translation at a downstream ATG codon is relatively rare in vertebrates, but has been demonstrated for a limited number of cellular and viral mRNAs (see references 15 and 26 for a review). Direct sequencing of the amino terminus of MxB protein is necessary to definitively establish its primary structure.

The Mx proteins of humans and mice have overall similarities of between 56 and 77%. A pairwise comparison reveals that the human MxA and the mouse Mx2 are most closely related proteins (Fig. 8). MxA protein shows a much lower degree of similarity to the human MxB and the mouse Mx1 proteins. This indicates that human MxA and mouse Mx2 proteins are encoded by homologous genes. However, MxB protein is probably not the true counterpart of mouse Mx1 protein, mainly for two reasons: MxB and mouse Mx1 proteins show a rather low degree of sequence similarity, and MxB is a cytoplasmic protein, whereas mouse Mx1 is a nuclear protein (5). It will be interesting to learn about the relationships of the two human Mx proteins to the two cytoplasmic rat proteins (19).

We have recently managed to establish several stable lines of transfected 3T3 cells that express high levels of either MxA or MxB protein in a constitutive manner. We will show elsewhere (J. Pavlovic, T. Zürcher, and P. Staeheli, submitted for publication) that transfected cell lines expressing human MxA protein have acquired a high degree of resistance to influenza virus, whereas cells expressing MxB protein do not show increased influenza virus resistance.

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