Human MxB Protein, an Interferon-α-inducible GTPase, Contains a Nuclear Targeting Signal and Is Localized in the Heterochromatin Region beneath the Nuclear Envelope*

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Interferon-inducible Mx proteins belong to the family of large GTPases and are highly homologous with dynamins within their GTP-binding domain. Cytoplasmically localized human MxA protein mediates resistance to influenza and several other viruses, whereas human MxB protein has not been found to have any antiviral activity. Here we show that MxB protein is found both in the cytoplasm and in the nucleus, where it is localized in a granular pattern in the heterochromatin region beneath the nuclear envelope. Transfection experiments in COS cells of N-terminally deleted MxB constructs revealed a functional nuclear localization signal within the first 24 N-terminal amino acids. Nuclear 78-kDa and cytoplastic 76-kDa forms of MxB protein were found in all of the cell lines studied and in human peripheral blood mononuclear cells. MxB protein proved to be a functional GTPase with activity comparable to that of MxA protein. N-terminally truncated (Δ1–92) MxB protein lacking both the nuclear localization signal and a proline-rich domain had almost completely lost its GTPase activity. Analysis of peripheral blood mononuclear cells suggested that MxB protein expression is strictly regulated by interferon-α. This is the first documentation that human Mx protein resides in the nucleus. It also emphasizes that there are considerable differences in the localization and structure of functional domains within Mx proteins.

The superfamily of GTPases includes several enzymes with important cellular functions both in procaryotes and in eucaryotes (1, 2). Several yeast, plant, and animal proteins, including VPS1 protein, MGM1 protein, phragmoplastin, Drosophila shibire protein, dynamins, and Mx proteins, have been grouped into the family of large GTPases (kDa ~70–100), whose GTP-binding elements are situated in the N-terminal part of the molecule, the remaining being responsible for other functions. Of these, VPS1 protein of Saccharomyces cerevisiae appears to play a direct role in the retention of proteins in the trans Golgi (3, 4). Yeast MGM1 protein is required for the integrity of mitochondrial genes (5), phragmoplastin is a dynamin-like protein associated with cell plate formation in plants (6), and D. shibire protein is involved in the recycling of synaptic vesicles and in the scission of clathrin-coated and non-clathrin-coated pits from the plasma membrane (7). Dynamin regulates coated vesicle endocytosis in cells (8, 9). It has been shown that dynamin can self-assemble and adopt a helical structure in solution, enabling it to wrap around the neck of membrane vesicles during vesicle formation from the plasma membrane (10–12). Nakayama et al. (13) have described an oligomerization motif in mouse Mx1 protein, located within the N-terminal GTP binding domain of the protein. This domain, which is also found in other large GTPases, is referred to as a dynamin family signature and may represent a self-assembly motif in Mx proteins. In addition, Mx proteins have a C-terminal leucine zipper domain which is capable of oligomerizing Mx proteins and heterologous proteins (14).

Sequence data from at least eight different vertebrate species reveals considerable conserved features in Mx proteins. All Mx proteins have a tripartite GTP binding domain in the N-terminal third of the protein (15). Mx proteins have an intrinsic GTPase activity ranging from 3 to 70 min⁻¹ (16–20). Although both Mx proteins and dynamins have a relatively high rate of hydrolysis, microtubules stimulate this activity in dynamins (21). Small GTPases (kDa ~20–35) such as p21H-ras, however, show an extremely low hydrolysis rate in the absence of GTPase-activating protein (1, 2). In general, GTPases are believed to act as molecular switches, the active state being associated with GTP binding (1). Several Mx proteins, such as human MxA protein and mouse and rat Mx1 proteins, have been shown to inhibit the replication of different types of RNA viruses, such as influenza, vesicular stomatitis, measles, and bunyaviruses (15). The antiviral activity of Mx proteins requires an intact GTPase domain and activity (18, 22). The expression of Mx proteins is strictly controlled by type 1 interferons (IFNs) or viruses (15, 23–25), and it has been suggested that Mx proteins form the first line of defense against viral infections, such as influenza virus infection (15, 25), before other parts of the immune system are activated.

In the present study we analyze the expression and subcellular localization of human MxB protein. We show that there are both nuclear and cytoplasmic forms of MxB protein, the former existing in granules localizing in the heterochromatin region beneath the nuclear envelope and having a specific

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** The abbreviations used are: IFN, interferon; NLS, nuclear localization signal; PRD, proline-rich domain; PBMC, peripheral blood mononuclear cell; PAGE, polyacrylamide gel electrophoresis; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; BSA, bovine serum albumin; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; bp, base pair(s); kb, kilobase pair(s).
N-terminal nuclear localization signal (NLS). We also demonstrate that MxB protein has GTPase activity comparable to that of MxA protein and that the N-terminal proline-rich domain (PRD) is likely to affect the GTPase activity of the molecule. The relatively high expression level of MxB protein in IFN-α-induced human leukocytes may be indicative of important cellular functions.

MATERIALS AND METHODS

Cells, Cultures, and Reagents—Monolayers and suspension cultures of Spodoptera frugiperda Sf9 cells were maintained in TNM-FH medium as described previously (26). Primary human leukocytes were obtained from healthy blood donors from the Finnish Red Cross Blood transfusion Service and were prepared and purified as described elsewhere (29). radioactive [γ-32P]ATP (3000 Ci/mmol) and [γ-32P]ATP (5000 Ci/mmol) were obtained from Amersham Corp.

Plasmids and DNA Manipulations—MxA cDNA was modified as described elsewhere (24). The noncoding sequences of the MxB cDNA were removed by PCR (30) by creating unique BamHI restriction sites immediately upstream of the first ATG codon and downstream of the STOP codon. The primers used were CAG GCG GAT CCA TCA TTA GGG CCA CAA GGA TGG G-G (5′) and GCC ATG GAT CCA TGG TAG TGG ATC TCG TTG CTG GAT AAT TG-3′ (the BamHI sites are in parentheses, the initiation and the STOP codons are underlined). The PCR product was digested with BamHI and, into a BamHI site of the pBc12 CMV (32), a transient expression vector that has been modified in our laboratory (22), to produce a plasmid pBc12CMV-MxB. To create a non-terminal MxB deletion constructs, we used PCR to insert into BamHI sites immediately upstream of the second, third, and fifth ATG codons of the MxB cDNA (see Fig. 4). The primers used were TAC CAG GCC GTG GAA ATG ATC TGG CAG CAA GCC CAA CCT CCA AAC TGG CAG, and ACC AAG GAT CAT GGC CCA TGG GGC CCA AGA ACA ACC TGG AC (the BamHI sites are in parentheses and the initiation codons are underlined). The primers to create BamHI sites for the 3′ ends was as described above. All of the PCR products were subcloned into pGEM3zf(+) vector (Promega, Madison, WI) to produce a plasmid pGEM3zf(+)-MxB. After sequencing the 5′ end of the MxB cDNA, human glioblastoma T98G (ATCC CRL 1690), COS-1 cells, and Vero (ATCC CCL 81), primary human peripheral blood mononuclear cells, and recombinant baculovirus-infected cells expressing Mx or MxA proteins.

Indirect Immunofluorescence Microscopy—For indirect immunofluorescence microscopy, the cells were grown on glass coverslips, either treated with IFN-α (1000 IU/ml) or left untreated, and then fixed with 3% paraformaldehyde and 1% glutaraldehyde (in PBS containing 0.1% Triton X-100). The cells were permeabilized in 0.1% Triton X-100 in PBS for 15 min. The cells were then treated with 0.5% BSA in PBS for 30 min. The cells were stained with guinea pig anti-MxA or anti-MxB antibodies at 1:500 to 1:2000 dilutions in PBS containing 0.5% BSA. The coverslips were washed with PBS, then twice with distilled water containing 0.05% Tween 20, and mounted in 25% Mowiol (Polysciences, Warrington, PA) in 25 mm Tris-HCl, pH 7.5, 50% glycerol, and 2.5% 1,4-diazabicyclo[2.2.2]octane (Scherger and Plough, Sandoz). Polymerized blocks were sectioned on a microtome with a diamond knife. 80-nm thin sections were picked up on Formvar-coated 200 mesh nickel grids, blocked with 0.5% BSA in PBS-T (10 μm Tris-HCl, pH 7.4, 20 μm NaCl, and 0.05% Tween 20) and then incubated in PBS-T and 0.5% BSA for 2 h at 37 °C with guinea pig anti-MxA antibodies (gift of Dr. R. Cantell of our institute (28). Human IFN-γ (1 × 106 IU/ml) was obtained from the Finnish Red Cross Blood transfusion Service and was prepared and purified as described elsewhere (29). Radioactive [γ-32P]ATP (3000 Ci/mmol) and [γ-32P]ATP (5000 Ci/mmol) were obtained from Amersham Corp.

Immunoelectron Microscopy—Immunoelectron microscopy was carried out on PBMCs treated with IFN-α (1000 IU/ml) for 48 h. After washing with PBS the cells were fixed with 3% paraformaldehyde and 1% glutaraldehyde (electrophorelograph grade, Sigma) in PBS in pellet form in Eppendorf tubes. After dehydration in a graded ethanol series the cells were mounted in LR White resin (36). Polymerized blocks were sectioned on a microtome with a diamond knife. 80-nm thick sections were picked up on Formvar-coated 200 mesh nickel grids, blocked with 0.5% BSA in PBS-T (10 μm Tris-HCl, pH 7.4, 20 μm NaCl, and 0.05% Tween 20) and then incubated in PBS-T and 0.5% BSA for 2 h at 37 °C with guinea pig anti-MxB antibodies (dilution, 1:200), followed by washing twice with PBS-T, and incubation with 5-nm gold-conjugated protein A (Molecular Probes, Eugene, OR). The sections were then treated with 0.5% BSA in PBS for 30 min. The sections were stained with 5% uranyl acetate for 10 min and 2% lead citrate for 3 min (Carlson’s method; LKB). The specimens were viewed on a Jeol JEM-1200EX transmission electron microscope at 60 kV. Control cells were manipulated as described, but stained with preimmune guinea pig serum.

Gel Electrophoresis and Western Blotting—Gel electrophoresis and Western blotting of MxA and MxB proteins were performed as described elsewhere (14, 22).

RNA Isolation and Analysis—PBMCs from five individuals were induced separately in six-well plates in RPMI 1640 medium with 5% FCS in 2 × 106 cells/ml, 300 IU/ml IFN-α and pooled before RNA isolation. At time points indicated in Fig. 7, the cells were harvested, and total RNA was analyzed as described previously (24), using MxB cDNA as a specific probe.

Primary Extension Assay—PBMCs from two individuals or HEp-2 cells were induced with IFN-α (1000 IU/ml) or left uninduced. At 6 h postinduction the cells were harvested, and total RNAs were collected using TRIzol reagent system (Life Technologies, Inc.). Primer extension assays were carried out with [γ-32P]ATP (Amersham Corp.) end-labeled oligonucleotides complementary to the region from +94 to +122 (5′-GAGGCCCCAGCTGAGGACGTGGCACTCAAGGAAG-3′) downstream of the first ATG codon using the avian myeloblastosis virus reverse transcriptase primer extension system (Promega). The amount of RNA used in a reaction was 10 μg (PBMC) or 75 μg (HEp-2). The primer extension products were electrophoresed on 8% polyacrylamide gel containing 7 M urea and autoradiographed.
The subcellular localization of MxB protein was examined in proteins are localized in the cell cytoplasm (23, 37). In this work, it has been reported that both human MxA and MxB proteins are arginine- and/lysine-rich sequences in a proper sterical context (38). A computer search of the MxB sequence revealed specific NLS in the protein molecule. Typically, NLS signals are arginine- and lysine-rich sequences in a proper sterical context, and the geometric mean fluorescence was analyzed separately for them.

**GTtase Activity Measurements of Ms Proteins**—The GTtase activity of MxA and MxB proteins was analyzed on immunoprecipitates of recombinant, baculovirus-infected *S. frugiperda* cells as described elsewhere (19, 22).

**RESULTS**

Purification of Baculovirus-expressed MxB Protein and Preparation of Specific Antisera—To obtain MxB protein for antibody production and for biochemical characterization, we inserted human MxB cDNA into a baculovirus expression vector. Because of its strong tendency to form aggregates, MxB protein was purified on a preparative SDS-PAGE for antibody production. To minimize any cross-reaction with MxA protein, the anti-MxA antibodies were absorbed with the MxA protein expressing recombinant baculovirus-infected cells and vice versa. The specificity of the antibodies as determined by indirect immunofluorescence microscopy (Figs. 1, 2, and 4) and Western blotting (compare lanes MxB, MxB(83–716), and MxA in Fig. 5) was very good, and no cross-reactivity was seen. The guinea pig antibodies used also showed clearly differential staining of MxA and MxB proteins in human macrophages (Fig. 2). Homologous absorption of anti-MxB and anti-MxA antisera with baculovirus-expressed MxB and Mxa proteins, respectively, resulted in a marked decrease of MxB- and MxA-specific signals in flow cytometric analysis (see Fig. 9, A and B; Abs), further supporting the specificity of the antibodies.

**Subcellular Localization of Human MxB Protein**—Previously it has been reported that both human MxA and MxB proteins are localized in the cell cytoplasm (23, 37). In this work the subcellular localization of MxB protein was examined in IFN-α-treated (1000 IU/ml) A549, HEp-2, T98G, and PBMC cells. It was also compared to that of Mxa protein. Surprisingly, immunofluorescence analysis revealed that, in all the types of cells tested, MxB protein was clearly localized with a granular pattern both in the nucleus and in the cytoplasm, whereas MxA protein was found only in the cell cytoplasm (Figs. 1 and 2). In established cell lines MxA protein was expressed in A549 and T98G cells, but was absent in HEp-2 cells. MxB protein, in contrast, was extremely poorly expressed in A549 cells, but was readily detected in HEp-2 and T98G cells (Fig. 1). After IFN-α induction, strong MxA protein expression was seen in the cytoplasm of PBMCs (Fig. 26), whereas MxB protein staining was observed both in the nucleus and in the cytoplasm (Fig. 2d). Differential focusing demonstrated an extremely granular fluorescence pattern with a tendency for MxB protein to be localized on the inner nuclear membrane (Fig. 2, e and f).

For immunoelectron microscopical experiments, PBMCs were treated with IFN-α (1000 IU/ml) for 48 h or left untreated, the cells were fixed and processed for immunoelectron microscopical staining with guinea pig anti-MxB serum, followed by staining with 5-nm gold-conjugated protein A. The nuclear form of MxB protein was found as large structures apparently localized in the heterochromatin region beneath the nuclear envelope (arrows in Fig. 3, a and b) but was almost always absent in nuclear pores (NP, open arrows in Fig. 3b). There was also specific staining within other parts of the nucleus and throughout the cytoplasm (arrows in Fig. 3, a and b). The cytoplasmic MxB-specific staining was often associated with membrane structures but clusters of MxB immunoreactive material were also found to be non-membrane-associated. The preimmune control serum revealed no specific staining (Fig. 3c).

**MxB Protein Has an N-terminal Nuclear Targeting Signal**—As human MxB protein was targeted into the cell nucleus in all the cells tested we wanted to study whether there was a specific NLS in the protein molecule. Typically, NLS signals are arginine- and lysine-rich sequences in a proper sterical context (38). A computer search of the MxB sequence revealed an N-terminal domain enriched with arginine-lysine residues (9 out of 25 amino acids; Fig. 4) that could function as an NLS. This domain was very hydrophilic and could thus be on the surface of the molecule. A cluster of basic residues was also observed at the very C-terminal end, but was very similar to the sequences of human MxA and rat Mx2 and Mx3 proteins which are localized in the cell cytoplasm. As some Mx protein
Fig. 2. Localization of MxA and MxB proteins in human primary macrophages detected by indirect immunofluorescence microscopy. The cells were induced with IFN-α (24 h, 1000 IU/ml) (b and d–f) or left uninduced (a and c). Staining was performed with guinea pig antibodies against MxA (a and b) and MxB (c–f) proteins, followed by staining with FITC-labeled anti-guinea pig immunoglobulins. MxB protein-specific staining was with longer (d) and shorter exposures (e and f) and MxA protein-specific staining (b). The fluorescent signal of MxB protein forms a discontinuous ring when the focal plane passes through the center of the nucleus (b). Uninduced control cells (a and c) were stained with anti-human MxA and MxB antisera, respectively. Bar, 10 μm.

FIG. 3. Immunoelectron microscopic localization of MxB protein in PBMCs. The cells were induced with IFN-α at 1000 IU/ml for 24 h (a–c), collected, fixed, and processed for immunoelectron microscopy. The sections were immunostained with guinea pig anti-MxB antiserum, followed by 5-nm gold-conjugated protein A. Gold particles are localized in the chromatin structures underlying the nuclear envelope (arrows in a and b). Control cells (c) were stained with preimmune guinea pig serum. N, nucleus; C, cytoplasm; NP, nuclear pores (open arrows); MxB-specific staining is indicated by small arrows. Bar, 200 nm.

Two Forms of MxB Proteins Are Expressed in Human Cells—Western blotting analysis was carried out to determine the molecular weights of MxB proteins produced by various cell lines and PBMCs. In all the types of cells studied, IFN-α induced the expression of MxB proteins of two different molecular masses, namely 78 and 76 kDa. As compared to the baculovirus-expressed marker proteins MxB and MxB(83–716), the 78-kDa protein represented the full-length translation product of the MxB gene with an NLS, whereas the 76-kDa protein matched to a molecule that was being translated from the second methionine (Fig. 5). In all the cell lines the 78-kDa protein constituted approximately 25% of the total MxB protein immunoreactivity. In the case of PBMCs and T98G cells, there were detectable basal levels of MxB protein without any IFN-α induction. It is significant to observe that the stable MxB-expressing Vero E6 cell line produced predominantly the 78-kDa form of MxB protein and hardly any 76-kDa protein, thus further supporting the idea that the 78-kDa protein is the one that is nuclearly targeted (Fig. 4f). All cells except HEp-2 appeared to express MxA protein in response to IFN-α induction (compare Figs. 1e and 5).

Structural and Functional Characteristics of MxB Protein—To test whether MxB protein is a functional enzyme with GTPase activity, we expressed the nuclear MxB protein and the cytoplasmic MxB(83–716) protein lacking both the NLS and proline-rich domains using the baculovirus expression system. We used immunoprecipitation with guinea pig anti-MxB and anti-MxA antisera to enrich the proteins (Fig. 6, A and B) and analyzed their GTPase activities in immunoprecipitates. We could not purify MxB protein by column chromatography, since both the full-length and truncated MxB proteins had a strong tendency to form aggregates during the purification procedures (results not shown). The relative GTPase activities of MxB and MxA protein were nearly the same (Fig. 6, C and D), indicating that, like other Mx proteins analyzed so far (murine Mx1 and human MxA proteins), MxB protein can hydrolyze GTP and is thus a functional GTPase. Of interest was the observation that the N-terminally truncated MxB(83–
716) protein had lost nearly all of its GTPase activity, as compared to wild type MxB protein where only 10% of the GTPase activity remained.

Cytokine Regulation of MxB Gene Expression in Human Blood Mononuclear Cells—In established cell lines both the MxA and MxB genes appear to be under the transcriptional
control of IFN-α (23, 40). To study the regulation of MxB gene expression specifically in primary human cells we stimulated PBMCs with high doses of IFN-α (300 IU/ml) and collected the cells at different periods of time after stimulation. Total cell RNA was isolated, and the kinetics of MxB mRNA expression was analyzed by Northern blotting. MxB mRNA levels rose relatively rapidly after IFN-α stimulation and remained at high levels up to 42 h poststimulation (Fig. 7A). Surprisingly, the kinetics of MxB mRNA expression was different from that of MxA mRNA, which gained basal levels somewhat faster, within 24 to 48 h postinduction (24). This suggests that MxB mRNA may have a longer half-life than MxA mRNA. Many IFN-inducible genes, such as the IRF-1, OAS, and MHC genes, are also activated by other cytokines (41). To test whether a selected set of other cytokines could activate MxB gene expression we stimulated PBMCs with interleukin-6 and tumor necrosis factor-α (TNF-α) alone or in combination with IFN-α for 4 h as indicated in the figure. Northern blots for MxB and β-actin mRNA are shown (B).
from that of cytoplasmic MxA protein (Figs. 1 and 2). Immunoelectron microscopic experiments demonstrated that MxB protein is found as large structures localized in the heterochromatin region beneath the nuclear envelope. This may suggest that MxB protein has some, as yet unknown, regulatory functions in the nucleus. It is unlikely that MxB protein regulates nuclear transport since the protein was very seldom found in nuclear pores. In the cytoplasm, clusters of MxB immunoreactive material were found within the membrane structures, whereas other clusters were non-membrane-associated (Fig. 3, a and b). Our observation of MxB protein in the nucleus differs from previous findings (37). These differences may be due to the cell lines (mouse instead of human) used, the quality of antibodies and the different cDNA constructs used to prepare stable cell lines. In natural situations, such as in primary human macrophages, MxB protein is indeed found in the nucleus.

In Western blot analysis, two MxB protein-specific bands, 78 and 76 kDa in size, were found (Fig. 5). Based on their apparent mobility in SDS-PAGE and on comparison with the baculovirus-expressed MxB and MxB(83–716) proteins, we suggest that the translation of the 78- and 76-kDa proteins starts from the first and second AUG codons of the same MxB mRNA, respectively (Fig. 5). In PBMCs, only one species of MxB mRNA was found (Fig. 7), consistent with studies carried out in other types of cells (23). Our primer extension analysis (Fig. 8) also supports the theory that the transcription of the MxB gene is initiated at one single site. The reason for the production of two forms of MxB protein is probably that the −3′/+4 nucleotide context CACATG of the first ATG codon is not optimal for the initiation of translation (42), and therefore translation is apparently also initiated from the second ATG codon. No proteolytic processing of the large 78-kDa MxB form was seen, which further support the theory that the two forms are direct translation products. One-fourth of the MxB protein produced seemed to be of the 78-kDa form, with the remainder representing the 76-kDa form (Fig. 5). It is significant to observe that, in the MxB-expressing Vero E6 cell line, the nuclear 78-kDa form of MxB protein was almost exclusively produced. This is possibly due to the fact that the MxB cDNA was manipulated by PCR immediately upstream of the first ATG codon to give a potentially better initiation of translation (CACATG changed to ACCATG).

To identify the possible NLS of MxB protein we transiently expressed the wild type and several N-terminally deleted MxB cDNA constructs in COS-1 cells. We found the translation product of full-length wild type MxB cDNA to be localized both in the nucleus and cytoplasm in a strongly granular staining pattern (Fig. 4a). All truncated MxB proteins remained in the cytoplasm (Fig. 4, b–d). In the full-length wild type 78-kDa MxB protein, the first 25 amino acids are rich in arginines and lysines, which apparently constitute the nuclear targeting signal of the protein (Fig. 4a). NLSs have been shown to be recognized by specific binding proteins hypothesized to have a receptor/carrier role in the active transport of NLS-containing proteins into the nucleus (38). The nuclear localization signal must also be properly exposed for transport to occur. The first 25 amino acids of the 78-kDa MxB protein are very hydrophobic. Computer analysis gave a very high surface probability for this region which would thus be readily exposed to NLS-binding proteins. It is interesting to observe that the nuclear localization signal of murine Mx1 protein is at the very C-terminal end of the protein (14, 43). Computer analysis also suggested this region to be on the surface of the protein. Duck Mx protein is also partly found in the nucleus (44), although its nuclear localization signal has not been identified. Murine Mx1 protein

**DISCUSSION**

In the present work we have characterized the expression, structure, and subcellular localization of the other human Mx protein, MxB protein. Surprisingly, we found that in IFN-α-treated lung carcinoma A549, epidermoid carcinoma HEp-2, glioblastoma T98G, or PBMCs, MxB protein was observed as large granules in both the nucleus and the cytoplasm. The subcellular localization of MxB protein was clearly different...
Nuclear Localization of Human MxB Protein

All known Mx proteins have a conserved C-terminal leucine zipper domain, which, at least in the case of murine Mx1, mediates the oligomerization of the protein in vitro (14). In many regulatory and structural proteins, a leucine zipper functions as a common oligomerization element (45). We also found human MxB protein to form oligomers as analyzed by chemical cross-linking in vivo (results not shown). There are certain differences in the leucine repeats of MxB protein as compared to other Mx proteins (Fig. 4). There are phenylalanine and isoleucine residues instead of leucines in the first and fourth positions, respectively, suggesting that the capacity of MxB protein to oligomerize could be weaker than that of MxA protein. However, we have demonstrated that a shorter three-leucine C-terminal tail was sufficient to cause oligomerization (19). A leucine zipper element may also mediate the dimerization of different proteins. For instance, Fos and Jun transcription factors, which contain leucine zipper motifs, form heterodimers (46). MxA and MxB proteins possibly do not form any heterooligomers in PBMCs or in other cell lines, since MxA protein was found only in the cytoplasm (compare the different staining pattern of MxA and MxB protein in Figs. 1 and 2). If MxA/MxB complex were formed, some MxA protein could be translocated into the nucleus together with MxB protein. In none of the studied cells was this the case.

In Fig. 10 we schematically summarize the current knowledge of species-specific Mx protein structure-function relationships. Human MxB protein is clearly different from most other Mx proteins, since it contains an N-terminal NLS and a domain rich in prolines (Figs. 4 and 10). PRDs are common in many functionally diverse proteins, including certain structural proteins, actin-binding proteins, and transcription factors. They are believed to have conformationally restricted structures that provide sites for interactions (e.g. Src-homology domains; SH3) with other molecules (47). In vitro analyses have shown that dynamin GTPase activity is stimulated by cross-linking with microtubules through the C-terminal proline-rich domain (39). Using COS-1 cell transfections and indirect immunofluorescence microscopy, we observed that the wild type 78-kDa MxB protein and the two deletion mutant proteins with an intact proline-rich domain (MxB(25–716) and MxB(25–716)) were found as granules in the cytoplasm, whereas the MxB(83–716) protein lacking the entire PRD was found in a diffuse manner throughout the cytoplasm. At present, there is no evidence that the proline-rich regions of the Mx proteins (although not representing a PXXP consensus pattern) mediate protein-protein interactions with other heterologous molecules but they clearly affect the clustering of MxB protein in cells.
analyzed their GTPase activity. The relative GTPase activities of MxA and MxB protein were nearly the same. Surprisingly, the N-terminally truncated Mx B (83–716) protein had lost nearly all of its GTPase activity. This suggests that either the deletion resulted in misfolding of MxB (83–716) protein or that the proline-rich domain has an important role as an element enhancing GTPase activity. Observations concerning the α-subunit of the heterotrimeric G protein have demonstrated that the GTP binding and GTP hydrolysis domains are clearly separate (48). Recent analysis of MxA protein has suggested that a C-terminal element of MxA protein is required for its GTPase activity (49), although the mechanism of MxB protein-induced GTP hydrolysis could be different. More detailed mutational analyses are needed to characterize the functional elements of MxB and other Mx proteins.

Although there has been great progress in understanding the structure and function of Mx proteins, their physiological role is still not known. Most mammalian species seem to have at least one Mx protein with antiviral activity. In addition, Mx proteins with no demonstrable antiviral activity have also been described (15). Based on the data described here we suggest that both nuclear and cytoplasmic Mx proteins are widely distributed among mammals. The nuclear localization signal is found either in the N-terminal (human MxB protein) or C-terminal (murine Mx1 protein) end of the protein (Fig. 10). Nuclear Mx proteins can also be differentially localized, e.g. associated with the heterochromatin beneath the nuclear envelope (human MxB protein), or seen as large aggregates (murine Mx1 protein) (19) with no apparent association with specific nuclear structures. The presence of Mx proteins in different subcellular compartments in different cell types in a number of vertebrates and the close relationship to other large GTPases, such as dynamin suggest that Mx proteins may have functions that are fundamental to cellular physiology in addition to their role in providing resistance to viral infections. The nuclear localization of Mx protein compels us to look more closely at the possible regulatory functions of these interesting proteins.

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