Interferon-Induced Guanylate-Binding Proteins Lack an N(T)KXD Consensus Motif and Bind GMP in Addition to GDP and GTP

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The primary structures of interferon (IFN)-induced guanylate-binding proteins (GBPs) were deduced from cloned human and murine cDNAs. These proteins contained only two of the three sequence motifs typically found in GTP/GDP-binding proteins. The N(T)KXD motif, which is believed to confer guanine specificity in other nucleotide-binding proteins, was absent. Nevertheless, the IFN-induced GBPs exhibited a high degree of selectivity for binding to agarose-immobilized guanine nucleotides. An interesting feature of IFN-induced GBPs is that they strongly bound to GMP agarose in addition to GDP and GTP agaroses but failed to bind to ATP agarose and all other nucleotide agaroses tested. Both GTP and GMP, but not ATP, competed for binding of murine GBP-1 to agarose-immobilized GMP. The IFN-induced GBPs thus define a distinct novel family of proteins with GTP-binding activity. We further demonstrate that human and murine cells contain at least two genes encoding IFN-induced GBPs. The cloned murine cDNA codes for GBP-1, an IFN-induced protein previously shown to be absent from mice of Gbp-1b genotype.

Guanine nucleotide-binding proteins play important roles in a large number of basic cellular functions such as protein synthesis, signal transduction, and intracellular protein transport (for a review, see reference 1). Most known guanine nucleotide-binding proteins contain a highly conserved sequence element which consists of the motifs GXXXXGKS(T), DXDG, and N(T)KXD (8, 9, 24). If present in a given protein sequence in an ordered fashion and with typical spacing, these motifs are highly indicative for associated guanine nucleotide-binding activity. In the most carefully studied guanine nucleotide-binding proteins, the human ras oncogene protein and elongation factor Tu of Escherichia coli, the first two consensus motifs constitute the phosphoryl-binding sequences, whereas the third element represents the guanine specificity region (10, 17, 23, 25). Accordingly, guanine nucleotide-binding proteins containing the first two consensus motifs but lacking a typical third motif exhibit decreased guanine specificity. Such proteins are kinesin (24), whose affinity for ATP is higher than that for GTP (7, 9, 19), and phosphoenolpyruvate carboxykinase (8), whose affinity for GTP is similar to that for ITP.

Guanine nucleotide-binding proteins are found among the many proteins whose synthesis is strongly stimulated in interferon (IFN)-treated cells. The IFN-induced Mx proteins are believed to exhibit GTP-binding activity since their sequences contain a perfect consensus element (15, 26, 29). Another IFN-induced protein, the cytoplasmic human 67-kDa guanylate-binding protein (GBP), designated hGBP-1, was identified by virtue of its very high affinity for agarose-bound GDP, GDP, or GTP (4). IFN-treated murine cells express several related proteins, namely, an abundant 65-kDa protein, designated mGBP-1, and a few less abundant proteins of smaller size (4, 32). Interestingly, some mouse strains lack the ability to synthesize GBP-1 in response to IFN. The ability of these mice to synthesize the smaller GBPs is not impaired, however (32). IFN-regulated synthesis of mGBP-1 is inherited as a single dominant genetic trait which mapped to the distal region of mouse chromosome 3 (27). Alleles Gbp-1a and Gbp-1b define inducibility and noninducibility, respectively, of murine GBP-1 synthesis, (32).

The physiological roles of the IFN-induced GBPs are not known. These proteins accumulate to high levels in IFN-treated cells and would thus qualify as potential intracellular mediators of the IFN-induced antiviral and antiproliferative effects (for a review, see reference 29). GBP-1a mice or cells derived from such mice are not more susceptible to viral infections or malignant transformation than are their Gbp-1a counterparts (27). This might indicate that the minor IFN-induced GBPs can functionally complement the lack of GBP-1.

To characterize the IFN-induced GBPs in more detail, we cloned and sequenced cDNAs encoding hGBP-1, its apparent isoform hGBP-2, and mGBP-1. We now report that human and murine GBPs contained the first two motifs of the guanine nucleotide-binding consensus domain but lacked the N(T)KXD guanine specificity motif. Nevertheless, the IFN-induced GBPs showed a high binding affinity for GDP and GTP as well as for GMP but failed to bind adenine, uracil, or cytosine nucleotides or 7-methyl-GMP. We further show that GBP-1 mRNA synthesis was impaired in Gbp-1b mice.

MATERIALS AND METHODS

IFNs and IFN inducers. Human IFN-α2 (10^6 U/ml) and human IFN-γ (10^7 U/ml) were purchased from Interferon Sciences, New Brunswick, N.J. IFN was induced in mice by intraperitoneal injections (1 ml per mouse) of buffered saline containing 100 μg of poly(I-C) (Sigma).

RNAs. Poly(A)^+ RNA was prepared from IFN-treated and control human FS-2 fibroblasts as described previously (4). Total RNA was prepared from mouse spleens as described by Chomczynski and Sacchi (5).
cDNA libraries. The murine cDNA library used in this study was previously described (30). A human cDNA library was prepared in lambda gt11 as follows. Samples (5 μg) of poly(A)⁺ RNA of IFN-γ-treated FS-2 cells were used for the synthesis of double-stranded DNA according to the procedure of Gubler and Hoffman (13). The cDNA was then ligated to EcoRI-cleaved, phosphatase-treated lambda gt11 arms and packaged by using packaging extracts from Stratagene Cloning Systems, San Diego, Calif.

cDNA library screening with antibody. The gt11 cDNA library was screened with rabbit antisera to the 67-kDa hGBP (2) as described by Hughn et al. (16), with some modifications. Briefly, about 10,000 recombinant phage clones were used to infect E. coli Y1090, and the cells were spread on 80-mm agar dishes. After a 6-h incubation at 42°C, the phage particles were lifted with colony/plaque screen hybridization transfer membranes (New England Nuclear). The membranes were washed with phosphate-buffered saline containing 0.2% Tween 20 (PBS-Tween), preincubated at room temperature for 2 h in PBS-Tween containing 5% nonfat dry milk, and incubated at room temperature for 2 h in the same solution containing rabbit antisera to the 67-kDa hGBP at a 1:500 dilution. The membranes were then washed with PBS-Tween, incubated for 1 h with biotinylated goat anti-rabbit immunoglobulin (Bethesda Research Laboratories), washed again, and incubated for 1 h with avidin-conjugated horseradish peroxidase (Vector Laboratories, Burlingame, Calif.). After being washed with PBS-Tween, the membranes were treated with freshly prepared substrate (0.06% 4-chloro-1-naphthol-0.012% hydrogen peroxide in PBS-Tween for 30 min) and developed with 0.06% 3,3'-diaminobenzidine-0.03% hydrogen peroxide in PBS-Tween for 15 min.

Screening of the murine cDNA library with an hGBP-2 cDNA probe. E. coli MC1061 was transfected with a sample of the amplified and size-selected plasmid library; ampicillin-resistant clones were transferred to nylon membranes, lysed, and hybridized to radiolabeled cDNA as described previously (30). The cDNA insert of an hGBP-2 phage served as a probe. Hybridization and washing of the membranes were performed at low stringency.

DNA sequencing. Sequencing was done by using Sequenase DNA polymerase according to the protocol of the enzyme manufacturer (United State Biochemical Corp., Cleveland, Ohio). Single-stranded M13-derived or double-stranded DNA templates were used.

hGBP-1 and hGBP-2 cDNA expression constructs. (i) pTZ18R34W. The EcoRI insert of hGBP-1 phase clone 34 was cloned into the EcoRI-cleaved and phosphatase-treated vector pTZ18, and its orientation was checked by sequencing over the junctions. This construct served as starting material for isolation of the GBP-1 promoter (21, 22).

(ii) pHG34S and pHG34AS. The EcoRI insert of hGBP-1 phase clone 34 was filled in with Klenow polymerase, ligated to BamHI linkers, and cloned in both orientations into the BamHI site of pHG327 (31).

(iii) pSP65mGBP-1. Because of the nature of the cDNA library used, the original mGBP-1 clone carried 12 extra G residues at its 5′ end, and therefore this cDNA needed some truncation at the 5′ end before it was suitable for RNA synthesis in vitro. Using polymerase chain reaction technology, we introduced a T-to-A mutation at position 29 of mGBP-1. This modification created a new EcoRI site in the 5′ noncoding region. Primers 5′-CCCTCCTAAGGAGATCC TCTTCAAGGAC-3′ (corresponding to positions 16 to 43) and 5′-GCCAGAGAGATCC TTCTCAAGGAC-3′ (corresponding to positions 184 to 163) were used to amplify mGBP-1 cDNA. Annealing was done at 37°C for 2 min, and DNA synthesis was done at 72°C for 3 min. After 25 cycles, the material was digested with EcoRI and BamHI, and the fragment corresponding to positions 28 to 150 of mGBP-1 was joined in a three-way ligation to the BamHI-HindIII fragment (positions 150 to 2805) of mGBP-1 and the EcoRI and HindIII-cleaved vector pSP65.

Transient expression of hGBP-1 in COS-7 cells. pHG34S or pHG34AS plasmid DNA was mixed to a final concentration of 2.5 μg/ml into serum-free medium containing DAE-dextran (0.5 mg/ml). Near-confluent COS-7 cells in 80-mm dishes were treated with 4 ml of this solution for 4 h at 37°C. The cells were washed with medium and further incubated for 3 h at 37°C in 2 ml of N-2-hydroxyethylpipеразине-N-2-ethanesulfonic acid (HEPES)-buffered saline containing 10% dimethyl sulfoxide. After being washed with medium, the cells were further incubated at 37°C for 24 h in medium containing 10% fetal calf serum. Labeling of the cellular proteins with [35S]methionine, cell lysis, and GMP agarose affinity chromatography were done as described previously for human fibroblasts (3, 4).

In vitro RNA synthesis. BamHI-linearized pTZ18R34W and HindIII-linearized pSP65mGBP-1 were used to direct capped RNA synthesis in vitro by using T7 and SP6 RNA polymerases, respectively, as suggested by the manufacturer of the enzymes (Promega).

In vitro synthesis of [35S]methionine-labeled proteins. Samples of synthetic RNAs were translated in vitro, using reticulocyte lysate containing [35S]methionine as suggested by the manufacturer of the kit (Promega).

Nucleotide agarose binding assays. Samples (2 μl) of reticulocyte lysates were prepared by adding radiolabeled GBPs were diluted with 70 μl of binding buffer (20 mM Tris-HCl [pH 7.0], 150 mM NaCl, 5 mM MgCl₂, 0.1% Triton X-100) and mixed with 30 μl of packed agarose beads equilibrated in the same buffer. After 30 min on ice, the beads were washed three times with 1 ml of ice-cold binding buffer, and bound protein was eluted by heating the sample for 3 min to 100°C in 50 μl of sample buffer containing sodium dodecyl sulfate (SDS). This material was analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) and autoradiography. For the competition studies, we mixed appropriate amounts of competitor with GMP agarose before adding the diluted reticulocyte lysate preparation containing mGBP-1.

Nucleotide sequence accession numbers. The three GBP cDNA sequences reported here have been deposited with GenBank under accession numbers M55542 (hGBP-1), M55543 (hGBP-2), and M55544 (mGBP-1).

RESULTS

Isolation of hGBP-1 and hGBP-2 cDNA clones. A lambda gt11 library prepared from mRNA of human fibroblasts treated with IFN-γ was screened with a polyclonal rabbit antisera to the 67-kDa GBP (3). Several positive recombinant phages were identified, and partial restriction maps of their cDNA inserts were established. Surprisingly, the antibody-reactive clones could be divided into two distinct classes, designated hGBP-1 and hGBP-2, on the basis of the presence or absence of an EcoRI site.

To determine whether the hGBP-1 and hGBP-2 clones were both derived from IFN-induced mRNAs, we hybridized a Northern (RNA) blot with mRNAs of IFN-treated and untreated control cells to the radiolabeled cDNA inserts of representative phages. hGBP-1 cDNA (originally designated p10 [3]) hybridized to an abundant 3.0-kb mRNA of IFN-treated human fibroblasts (Fig. 1). This mRNA was not
detectable in untreated control cells. hGBP-2 cDNA also hybridized to an IFN-induced mRNA, but this mRNA was less abundant and only about 2.2 kb long (Fig. 1). Rescreening of our lambda gt11 library with hGBP cDNAs as hybridization probes eventually led to the isolation of hGBP-1 and hGBP-2 phages with long cDNA inserts (about 2.9 and 1.9 kb, respectively). We concluded that our longest hGBP-1 cDNA (phage clone 34) was probably close to full length, whereas our longest hGBP-2 cDNA (phage clone 35) most likely represented an incomplete copy of the corresponding mRNA. In IFN-treated FS-2 cells, hGBP-2 mRNA was about 10-fold less abundant than hGBP-1 mRNA, as judged from the relative intensities of the Northern signals and from the number of hGBP-1 and hGBP-2 cDNA clones present in the library.

To determine whether the hGBP-1 cDNA of phage clone 34 indeed contained a functional open reading frame and whether the encoded protein would bind to guanine nucleotides, we cloned this cDNA in both orientations into the plasmid vector pTZ18 and synthesized capped RNAs in vitro by using T7 RNA polymerase. Figure 2A shows that the RNA of one of these clones was translated into a 67-kDa polypeptide that exhibited a high affinity for GMP agaroase. This protein was immunoprecipitated by our anti-GBP antiserum but not by the preimmune serum. To further demonstrate that the cloned hGBP-1 cDNA was coding for a functional protein, we inserted the cDNA into the eukaryotic expression vector pHG327 downstream of the simian virus 40 early promoter, transiently transfected COS-7 cells, and tested for the presence of GBP-1 in the cell lysates by GMP agaroase chromatography. Figure 2B shows that COS-7 cells transfected with the hGBP-1 sense construct contained a 67-kDa protein with GMP-binding affinity. COS-7 cells transfected with the hGBP-1 antisense construct lacked this protein. Taken together, these results indicated that the cloned cDNA was a functional copy of hGBP-1 mRNA.

Isolation of a murine cDNA clone related to hGBPs. Northern blot analysis indicated that cultured embryo cells from BALB/c mice contained IFN-induced mRNAs of about 3 kb length that weakly hybridized to the hGBP cDNA probes (data not shown). Screening of an appropriate murine cDNA library with radiolabeled hGBP-2 cDNA permitted the isolation of several cross-reactive clones, the longest of which (clone 8) had an insert of about 2.8 kb.

The mGBP cDNA clone codes for GBP-1, an IFN-induced protein not synthesized by mice of Gbp-1<sup>−</sup> genotype. To determine whether the murine GBP clone 8 might encode GBP-1 which is found in mice of Gbp-1<sup>−</sup> genotype only, we prepared a Northern blot of mRNAs from Gbp-1<sup>−</sup> (BALB/c and A/J) and Gbp-1<sup>−</sup> (A2G and C57BL/6) mice. The mice were treated for 6 h either with 100 μg of the IFN-inducing
from blot 3.0 and cDNA complete probe, hybridization IFN-induced 2.5-kb mRNA poly(I-C) sacrificed and total (Fig. 4. cDNA sequences genomic clone reprobed that clone 8A2G 83A). In Fig. 3B). The simplest interpretation of these results was that clone 8 was derived from the 3.0-kb mRNA and that the region of this RNA showed a high degree of sequence similarity to the 2.5-kb mRNA. The fact that the 3.0-kb mRNA was induced in poly(I-C)-treated Gbp-1+ but not Gbp-1− mice suggested that clone 8 indeed encoded GBP-1, an assumption that was confirmed later by cDNA expression experiments (see below). The 2.5-kb mRNA might code for the minor GBPs known to be present in IFN-treated cells of both Gbp-1+ and Gbp-1− genotypes (32).

A more careful examination of the Northern data revealed that the hybridization patterns of the two Gbp-1+ strains A2G and C57BL/6 were not identical. The 3.0-kb GBP-1 mRNA was completely absent from the spleens of C57BL/6 mice, whereas small but readily detectable amounts of this

FIG. 4. cDNA sequences and deduced protein structures of human and murine GBPs. The heteropolymeric sequences of hGBP-1 clone 34 (A) hGBP-2 clone 35 (B), and mGBP-1 clone 8 (C) are shown. The sequence of the 14 bases at the very 5' end of hGBP-1 cDNA was derived from a genomic clone (22).

substance poly(I-C) or with buffered saline before they were sacrificed and total spleen RNA was prepared. When the complete cDNA insert of the mGBP clone 8 was used as a hybridization probe, two distinct IFN-induced mRNAs of about 3.0 and 2.5 kb were detected in BALB/c and A/J spleens (Fig. 3A). In contrast, this probe detected the IFN-induced 2.5-kb mRNA but not the 3.0-kb mRNA in the spleens of A2G and C57BL/6 mice. When the same Northern blot was reprobed at high stringency with a radiolabeled restriction fragment derived from the 3' end of GBP clone 8, only the 3.0-kb mRNA but not the 2.5-kb mRNA was detected (Fig. 3B). The simplest interpretation of these results was that clone 8 was derived from the 3.0-kb mRNA...
mRNA were in the spleens of poly(I)-C-treated and untreated A2G mice (Fig. 3). In contrast to the situation in Gbp-1 stimulated, poly(I)-C treatment of A2G mice did not result in an increase of the GBP-1 mRNA pool, suggesting that A2G mice have a defective Gbp-1 promoter region. The two inbred mouse strains A2G and C57BL/6 thus define two distinct Gbp-1 alleles.

Sequence analysis of human and murine GBPs reveals a variant GTP-binding consensus domain. The complete cDNA structures of the hGBP-1 clone 34, hGBP-2 clone 35, and mGBP-1 clone 8 were established (Fig. 4). The heteropolymorphic sequence of hGBP-1 cDNA comprises 2,881 bases. The ATG codon at position 69 marks the beginning of a long open reading frame that encodes a 592-amino-acid protein with a calculated molecular weight of 67,902. The hGBP-1 cDNA sequence contains an in-frame termination codon at position 69, indicating that the ATG codon at position 69 indeed serves to initiate protein synthesis. The sequence of our longest hGBP-2 cDNA comprises 1,936 bases. An open reading frame extends from the very extreme 5' end to position 1758, suggesting that it does not contain the complete GBP-2 coding region. The sequence contains neither a polyadenylation signal nor a poly(A) tail and might therefore also be devoid of sequences corresponding to the 3' noncoding region of the GBP-2 mRNA. The heteropolymeric sequence of the mGBP-1 cDNA comprises 2,807 bases and is presumably a nearly full-length copy of mGBP-1 mRNA. The ATG at position 64, which is preceded by an in-frame termination codon, marks the beginning of an open reading frame that encodes the 589-amino-acid mGBP-1.

A homology search against the NBRF protein database, using the deduced protein sequences of hGBP-1 and hGBP-2 as query sequences, failed to reveal extensive homology to sequences of other known proteins.

The amino acid sequences of the two hGBPs and of mGBP-1 show a high degree of identity (Fig. 5). The overall similarity between hGBP-1 and mGBP-1 is about 70%; the two hGBPs are about 75% identical. The highest degree of identity is observed in the amino-terminal two-thirds of the three proteins, whereas the sequences of their carboxyterminal 25 to 30 amino acids are not related. The regions of strict sequence conservation include the sequences GLYRT-GKS and TDEG (underlined regions designated I and II in Fig. 5). These sequences perfectly match the first two motifs of the tripartite consensus sequence GXXXGK(S/T), DXG, N(T/K)X, which is found in a large number of proteins with GTP-binding activity (8). The spacing between the two motifs in all three GBPs is 44 amino acids, a distance

FIG. 4.—Continued.
similar to that of other known GTP/GDP-binding proteins. Interestingly, the IFN-induced GBPs lack the third motif, which is believed to determine the specificity for guanine nucleotides. Neither the sequence TKXD nor the reversed motif DXXK (or DXK) is found in the human or murine GBPs. The GBPs also lack a TQXD motif, which seems to function in place of the N(T)KXD motif in the human placental GTP-binding protein p25 (28).

The C-terminal four amino acids of the three IFN-induced GBPs are CTIS, CNIL, and CTIL, respectively (Fig. 5). Similar motifs, designated CAXX motifs, are found at the extreme C termini of the yeast mating factor, the H-ras oncogene protein, and some G proteins. These sequences may serve as a signal for posttranslational modification (6, 14, 33). The significance of the CAXX motif in the IFN-induced GBPs is not known.

**mGBP-1 strongly binds to GMP, GDP, and GTP but not to other nucleotides or mRNA cap structures.** Earlier experiments with partially purified hGBP-1 indicated a high specificity of this protein for guanine nucleotides; GTP and GMP, but not AMP or ATP, competed strongly for the binding of radiolabeled GTP to hGBP-1 (2). In light of the results presented above that the IFN-induced GBPs lack the N(T)KXD motif of the GTP consensus element, we reexamined their nucleotide-binding properties. Since the N(T)KXD motif is thought to determine guanine specificity, we assayed in vitro-synthesized human and murine GBP-1 for binding to a panel of 13 different agarose-coupled nucleotides. Under

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**FIG. 4—Continued.**
VOL. 11, are boxed. The corresponding given binding and washing conditions of the affinity chromatography material, more than 10% of the input mGBP-1 was found associated with the GMP, GDP, and GTP agarose beads (Fig. 6). GGBP-1 was recovered most efficiently by GDP agarose, but GTP and GMP agarose were also potent affinity matrices for mGBP-1. In contrast, less than 0.5% of the input material was retained by agarose beads loaded with AMP, ADP, ATP, CMP, CDP, CTP, UMP, UDP, or UTP. Similarly, mGBP-1 did not bind to agarose beads loaded with 7-methyl-GMP, an affinity matrix suitable for the isolation of mRNA cap-binding proteins. Indistinguishable nucleotide-binding data were obtained with hGBP-1 (data not shown).

To estimate the relative affinities of mGBP-1 to GMP and GTP, we performed a series of binding competition experiments. In vitro-synthesized radiolabeled mGBP-1 was reacted with GMP agarose in the presence of GMP, GTP, or ATP, and residual binding of mGBP-1 to the affinity matrix was monitored. GTP and GMP interfered with binding, whereas ATP was ineffective (Fig. 7). GTP and GMP inhibited the binding of mGBP-1 to GMP agarose in a concentration-dependent manner. Inhibition by GTP was most effective at 10 mM but was still observed at 0.3 mM. GMP was ineffective at 0.3 mM but inhibited binding of mGBP-1 to GMP agarose quite strongly at 10 mM. ATP had no inhibitory effect at all concentrations tested. Similar results were obtained when GTP or GDP agarose was used for affinity chromatography.

**DISCUSSION**

Using antibodies, we have cloned cDNAs encoding IFN-induced GBPs of human fibroblasts. Unexpectedly, we identified two rather than one species of GBP mRNAs encoding related proteins. The more abundant of these mRNAs (hGBP-1) most likely encodes the well-characterized 67-kDa GBP (2-4). The other mRNA (hGBP-2) is derived from a different gene and is about 10 times less...
The similarity of the deduced protein sequences suggests that hGBP-1 and hGBP-2 are isomers with similar biochemical properties.

A comparison of the IFN-induced GBPs and other known proteins with GTP-binding activity revealed some interesting features. As expected, IFN-induced GBPs contained some highly conserved sequence motifs, but surprisingly, only two of the three motifs typically found in GTP/GDP-binding proteins were present. GTP-binding consensus elements recognized to date consist of the three motifs GXXXXGXXS (T), DXXG, and N(T)KXXD (8, 9, 24). From the analysis of crystallized human ras oncogene protein and E. coli elongation factor Tu, it was concluded that conserved amino acids of the first two motifs interact with the β-phosphate of bound guanine nucleotides, whereas conserved residues of the third motif are in close contact with the guanine ring (20, 23, 25), indicating that the N(T)KXXD motif represents the guanine specificity region. Interestingly, the IFN-induced GBPs lacked the N(T)KXXD motif. However, when recombinant radiolabeled human and murine GBPs were tested for their nucleotide-binding specificities, a very strong selectivity for guanine mono-, di- and triphosphates was observed (Fig. 6). The IFN-induced GBPs failed to bind adenosine, uracil, or cytosine nucleotides. Thus, IFN-induced GBPs exhibit a high degree of guanine nucleotide-binding specificity despite lacking the N(T)KXXD guanine specificity motif. Another example of a GTP-binding protein that, like IFN-induced GBPs, contains the first two GTP-binding consensus motifs with typical spacing but lacks the third motif of the consensus sequence is kinesin (24). Unlike the IFN-induced GBPs, however, kinesin also binds ATP with high affinity (7, 19). Similarly, phosphoenolpyruvate carboxykinase, which lacks a perfect third GTP-binding consensus motif, shows poor specificity for guanine nucleotides (9). Our findings thus extend the established view about the role of the N(T)KXXD motif in GTP-binding proteins and suggest that unrelated sequences can equally well determine guanine specificity.

IFN-induced GBPs are further unique among known guanine nucleotide-binding proteins in that they strongly bind to GMP in addition to GDP and GTP. Although their affinity for GMP may be about 1 order of magnitude lower than for GTP, these observations still indicate that the phosphoryl-binding domain of the IFN-induced GBPs is more promiscuous than in most other proteins with GTP-binding activity. In analogy to the ras protein and elongation factor Tu, we assume that this domain might include the conserved GXXXXGXXS(T) and DXXG motifs. We do not know whether the nonconserved amino acids in the two consensus motifs or some sequences outside the motifs are responsible for the GMP-binding activity. To eventually define the structures required for guanylate binding, it will be necessary to generate crystallographic data of IFN-induced GBPs complexed with their different substrates.

Obtaining formal proof that proteins with GTP-binding consensus motifs indeed bind to guanine nucleotides was often difficult, and in some cases rather sophisticated assay systems had to be used (11, 12, 18). IFN-induced GBPs are exceptional in that their guanylate-binding activities can be assayed by affinity chromatography, a simple and straightforward technique. This fact will certainly facilitate future experimental identification of the domains of IFN-induced GBPs that are required for the specific binding of guanylates.

In this report we further characterize the murine GBP system, which is of particular interest because of its genetic.

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