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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-1*^{b} 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), DXXG, 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, 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 GMP, 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-1*^{a} and *Gbp-1*^{b} 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-1*^{b} mice or cells derived from such mice are not more susceptible to viral infections or malignant transformation than are their *Gbp-1*^{a} 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-1*^{b} mice.

MATERIALS AND METHODS

IFNs and IFN inducers. Human IFN-α2 (10^8 U/mg) and human IFN-γ (10^7 U/mg) 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).

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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 *Eco*RI-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 antiserum to the 67-kDa hGBP (2) as described by Hugnh 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 antiserum 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 color development.

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 mGBP-1 cDNA expression constructs. (i) pTZ18R34W. The *Eco*RI insert of hGBP-1 phage clone 34 was cloned into the *Eco*RI-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 *Eco*RI insert of hGBP-1 phage clone 34 was filled in with Klenow polymerase, ligated to *Bam*HI linkers, and cloned in both orientations into the *Bam*HI 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 *Eco*RI site in the 5′ noncoding region. Primers 5′-CTTCTCTAAAGGAATTC TCTTCAGAGAC-3′ (corresponding to positions 16 to 43) and 5′-CACCACCACAGGCTGTGTAATG-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 *Eco*RI and *Bam*HI, and the fragment corresponding to positions 28 to 150 of mGBP-1 was joined in a three-way ligation to the *Bam*HI-*Hin*dIII fragment (positions 150 to 2805) of mGBP-1 and the *Eco*RI and *Hin*dIII-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 DEAE-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-hydroxyethylpiperazine-*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 [^{35}S]methionine, cell lysis, and GMP agarose affinity chromatography were done as described previously for human fibroblasts (3, 4).

In vitro RNA synthesis. *Bam*HI-linearized pTZ18R34W and *Hin*dIII-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 [^{35}S]methionine-labeled proteins. Samples of synthetic RNAs were translated in vitro, using reticulocyte lysate containing [^{35}S]methionine as suggested by the manufacturer of the kit (Promega).

Nucleotide agarose binding assays. Samples (2 μl) of reticulocyte lysate preparations containing radiolabeled GBPs were diluted with 70 μl of binding buffer (20 mM Tris-HCl [pH 7.0], 150 mM NaCl, 5 mM $MgCl_2$, 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 antiserum 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 internal *Eco*RI 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

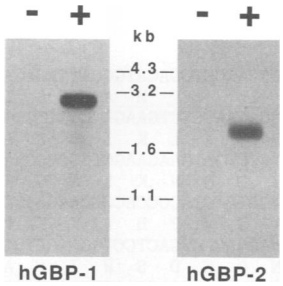

FIG. 1. Derivation of hGBP-1 and hGBP-2 cDNAs from distinct IFN-induced mRNAs. Northern blots were prepared from 0.5 μg of poly(A)$^+$ RNA from either untreated (−) human FS-2 fibroblasts or from cells treated for 5 h with 1,000 U of human IFN-α2 per ml (+). The blots were hybridized to radiolabeled probes derived from either hGBP-1 (positions 1 to 2881) or hGBP-2 (positions 1173 to 1936) cDNA. A DNA size standard was used to estimate the sizes of the target RNAs. The exposure times of the two blots were not identical.

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

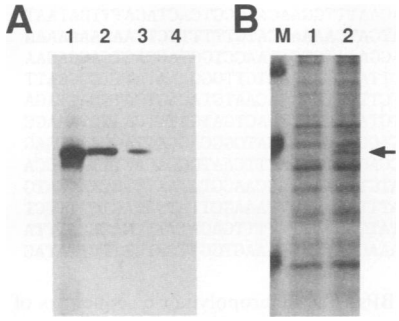

FIG. 2. Binding to GMP agarose of the protein encoded by hGBP-1 cDNA. (A) The hGBP-1 cDNA insert of phage clone 34 was cloned downstream of the T7 promoter. Capped RNA was synthesized from this construct (pTZ18R34W) and translated in vitro in the presence of [^{35}S]methionine. A sample of this material was analyzed by SDS-PAGE without further processing (lane 1). Other samples were subjected to GMP agarose affinity chromatography (lane 2), immunoprecipitated with a rabbit antiserum to hGBP-1 (lane 3), or immunoprecipitated with preimmune serum (lane 4). (B) The hGBP-1 cDNA insert of phage clone 34 was cloned in the antisense (lane 1) or sense (lane 2) orientation downstream of the simian virus 40 early promoter, and the constructs were used to transiently transfect COS-7 cells. At 24 h posttransfection, the cells were labeled with [^{35}S]methionine for 12 h, and extracts were prepared and subjected to GMP agarose affinity chromatography. The eluted material was analyzed by SDS-PAGE and autoradiography. The arrow marks the position of hGBP-1. Lane M, 95-, 68-, and 43-kDa protein size standards.

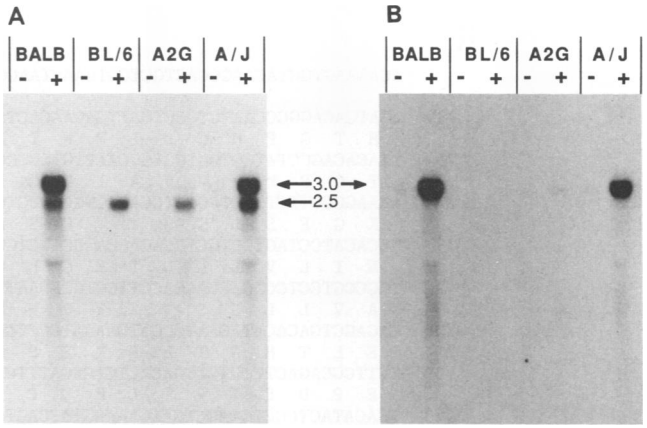

FIG. 3. Double-stranded RNA-induced accumulation of GBP mRNAs in the spleens of *Gbp-1^a* and *Gbp-1^b* mice. Mice of *Gbp-1^a* (BALB and A/J) and *Gbp-1^b* (BL/6 and A2G) genotypes were given intraperitoneal injections of either 100 μg of poly(I-C) in buffered saline (+) or buffered saline alone (−). The animals were sacrificed 6 h later, total spleen RNA was prepared, and 30-μg samples were subjected to Northern blot analysis. The blot was hybridized to the radiolabeled *Bam*HI-*Hin*dIII restriction fragment (positions 150 to 2805) (A) or the *Pst*I-*Hin*dIII restriction fragment (positions 2339 to 2805) (B) of mGBP-1 cDNA.

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 agarose. 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 agarose 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^b* genotype. To determine whether the murine GBP clone 8 might encode GBP-1 which is found in mice of *Gbp-1^a* genotype only, we prepared a Northern blot of RNAs from *Gbp-1^a* (BALB/c and A/J) and *Gbp-1^b* (A2G and C57BL/6) mice. The mice were treated for 6 h either with 100 μg of the IFN-inducing

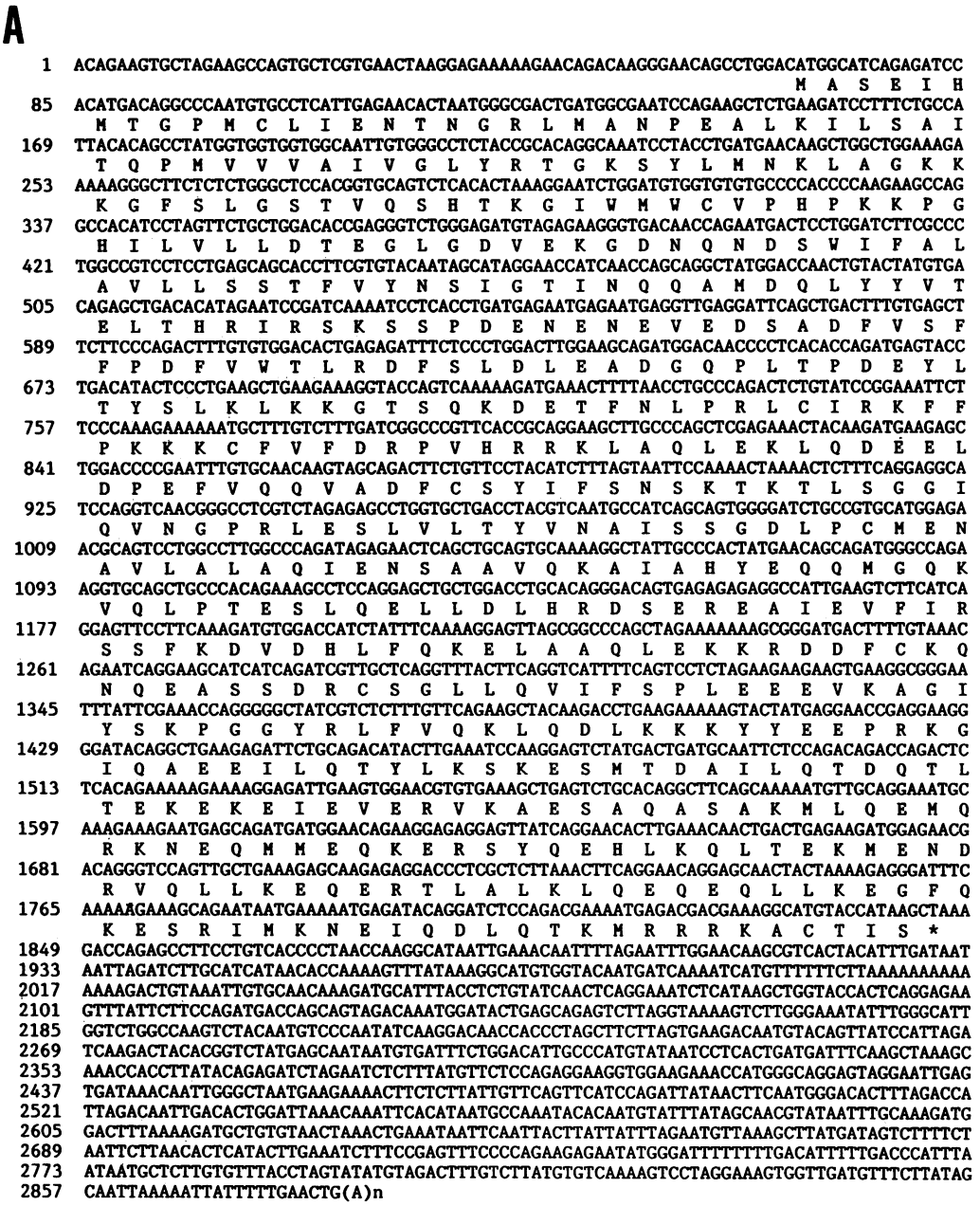

FIG. 4. cDNA sequences and deduced protein structures of human and murine GBPs. The heteropolymeric sequences of hGBP-1 clone 34 (A) hGBP-2 clone 35 (B), and mGBP-1 clone 8 (C) are shown. The sequence of the 14 bases at the very 5′ end of hGBP-1 cDNA was derived from a genomic clone (22).

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 and that the coding 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*^{a} but not *Gbp-1*^{b} 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*^{a} and *Gbp-1*^{b} genotypes (32).

A more careful examination of the Northern data revealed that the hybridization patterns of the two *Gbp-1*^{b} 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

B

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   1  CAACTTGCCGGGCCCAATGAGCCTATTGATAACACTAAAGGGCAGCTGGTGGTGAATCCAGAAGCTCTGAAGATCCTATCTGCA
      Q  L  A  G  P  N  E  P  I  D  N  T  K  G  Q  L  V  V  N  P  E  A  L  K  I  L  S  A
  85  ATTACGCAGCCTGTGGTGGTGGTGGCGATTGTGGGCCTCTATCGCACAGGCAAATCCTACCTGATGAACAAGCTGGCTGGGAAG
      I  T  Q  P  V  V  V  V  A  I  V  G  L  Y  R  T  G  K  S  Y  L  M  N  K  L  A  G  K
 169  AAAAACGGCTTCTCTCTAGGCTCCACAGTGAAGTCTCACACCAAGGGAATCTGGATGTGGTGTGTGCCTCATCCCAAGAAGCCA
      K  N  G  F  S  L  G  S  T  V  K  S  H  T  K  G  I  W  M  V  C  V  P  H  P  K  K  P
 253  GAACACACCCTAGTTCTGCTCGACACTGAGGGCCTGGGAGATATAGAGAAGGGTGACAATGAGAATGACTCCTGGATCTTTGCC
      E  H  T  L  V  L  L  D  T  E  G  L  G  D  I  E  K  G  D  N  E  N  D  S  W  I  F  A
 337  TTGGCCATCCTCCTGAGCAGCACCTTCGTGTACAATAGCATGGGAACCATCAACCAGCAGGCCATGGACCAACTTCACTATGTG
      L  A  I  L  L  S  S  T  F  V  Y  N  S  M  G  T  I  N  Q  Q  A  M  D  Q  L  H  Y  V
 421  ACAGAGCTGACAGATCGAATCAAGGCAAACTCCTCACCTGGTAACAATTCTGTAGACGACTCAGCTGACTTTGTGAGCTTTTTT
      T  E  L  T  D  R  I  K  A  N  S  S  P  G  N  N  S  V  D  D  S  A  D  F  V  S  F  F
 505  CCAGCATTTGTGTGGACTCTCAGAGATTTCACCCTGGAACTGGAAGTAGATGGAGAACCCATCACTGCTGATGACTACTTGGAG
      P  A  F  V  W  T  L  R  D  F  T  L  E  L  E  V  D  G  E  P  I  T  A  D  D  Y  L  E
 589  CTTTCGCTAAAGCTAAGAAAAGGTACTGATAAGAAAAGTAAAAGCTTTAATGATCCTCGGTTGTGCATCCGAAAGTTCTTCCCC
      L  S  L  K  L  R  K  G  T  D  K  K  S  K  S  F  N  D  P  R  L  C  I  R  K  F  F  P
 673  AAGAGGAAGTGCTTCGTCTTCGATTGGCCCGCTCCTAAGAAGTACCTTGCTCACCTAGAGCAGCTAAAGGAGGAAGAGCTGAAC
      K  R  K  C  F  V  F  D  W  P  A  P  K  K  Y  L  A  H  L  E  Q  L  K  E  E  E  L  N
 757  CCTGATTTCATAGAACAAGTTGCAGAATTTTGTTCCTACATCCTCAGCCATTCCAATGTCAAGACTCTTTCAGGTGGCATTGCA
      P  D  F  I  E  Q  V  A  E  F  C  S  Y  I  L  S  H  S  N  V  K  T  L  S  G  G  I  A
 841  GTCAATGGGCCTCGTCTAGAGAGCCTGGTGCTGACCTACGTCAATGCCATCAGCAGTGGGGATCTACCCTGCATGGAGAACGCA
      V  N  G  P  R  L  E  S  L  V  L  T  Y  V  N  A  I  S  S  G  D  L  P  C  M  E  N  A
 925  GTCCTGGCCTTGGCCCAGATAGAGAACTCAGCCGCAGTGGAAAAGGCTATTGCCCACTATGAACAGCAGATGGGCCAGAAGGTG
      V  L  A  L  A  Q  I  E  N  S  A  A  V  E  K  A  I  A  H  Y  E  Q  Q  M  G  Q  K  V
1009  CAGCTGCCCACGGAAACCCTCCAGGAGCTGCTGGACCTGCACAGGGACAGTGAGAGAGAGGCCATTGAAGTCTTCATGAAGAAC
      Q  L  P  T  E  T  L  Q  E  L  L  D  L  H  R  D  S  E  R  E  A  I  E  V  F  M  K  N
1093  TCTTTCAAGGATGTGGACCAAATGTTCCAGAGGAAATTAGGGGCCCAGTTGGAAGCAAGGCGAGATGACTTTTGTAAGCAGAAT
      S  F  K  D  V  D  Q  M  F  Q  R  K  L  G  A  Q  L  E  A  R  R  D  D  F  C  K  Q  N
1177  TCCAAAGCATCATCAGATTGTTGCATGGCTTTACTTCAGGATATATTTGGCCCTTTAGAAGAAGATGTCAAGCAGGGAACATTT
      S  K  A  S  S  D  C  C  M  A  L  L  Q  D  I  F  G  P  L  E  E  D  V  K  Q  G  T  F
1261  TCTAAACCAGGAGGTTACCGTCTCTTTACTCAGAAGCTGCAGGAGCTGAAGAATAAGTACTACCAGGTGCCAAGGAAGGGGATA
      S  K  P  G  G  Y  R  L  F  T  Q  K  L  Q  E  L  K  N  K  Y  Y  Q  V  P  R  K  G  I
1345  CAGGCCAAAGAGGTGCTGAAAAAATATTTGGAGTCCAAGGAGGATGTGGCTGATGCACTTCTACAGACTGATCAGTCACTCTCA
      Q  A  K  E  V  L  K  K  Y  L  E  S  K  E  D  V  A  D  A  L  L  Q  T  D  Q  S  L  S
1429  GAAAAGGAAAAAGCGATTGAAGTGGAACGTATAAAGGCTGAATCTGCAGAAGCTGCAAAGAAAATGTTGGAGGAAATACAAAAG
      E  K  E  K  A  I  E  V  E  R  I  K  A  E  S  A  E  A  A  K  K  M  L  E  E  I  Q  K
1513  AAGAATGAGGAGATGATGGAACAGAAAGAGAAGAGTTATCAGGAACATGTGAAACAATTGACTGAGAAGATGGAGAGGGACAGG
      K  N  E  E  M  M  E  Q  K  E  K  S  Y  Q  E  H  V  K  Q  L  T  E  K  M  E  R  D  R
1597  GCCCAGTTAATGGCAGAGCAAGAGAAGACCCTCGCTCTTAAACTTCAGGAACAGGAACGCCTTCTCAAGGAGGGATTCGAGAAT
      A  Q  L  M  A  E  Q  E  K  T  L  A  L  K  L  Q  E  Q  E  R  L  L  K  E  G  F  E  N
1681  GAGAGCAAGAGACTTCAAAAAGACATATGGGATATCCAGATGAGAAGCAAATCATTGGAGCCAATATGTAACATACTCTAAAAG
      E  S  K  R  L  Q  K  D  I  W  D  I  Q  M  R  S  K  S  L  E  P  I  C  N  I  L  *
1765  TCCAAGGAGCAAAATTTGCCTGTCCAGCTCCCTCTCCCCAAGAAACAACATGAATGAGCAACTTCAGAGTGTCAAACAACTGCC
1849  ATTAAACTTAACTCAAAATCATGATGCATGCATTTTTGTTGAACCATAAAGTTTGCAAAGTAAAGGTTAAGTATGAGGTCAATG
1933  TTTT
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FIG. 4—*Continued.*

mRNA were present in the spleens of poly(I-C)-treated and untreated A2G mice (Fig. 3). In contrast to the situation in *Gbp-1*^{a} mice, 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*^{b} 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 heteropolymeric 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 27, 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 near-full-length copy of mGBP-1 mRNA. The ATG at position 64, which is preceeded 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 data base, 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 carboxy-terminal 25 to 30 amino acids are not related. The regions of strict sequence conservation include the sequences GLYRT-GKS and DTEG (underlined regions designated I and II in Fig. 5). These sequences perfectly match the first two motifs of the tripartite consensus sequence GXXXXGKS(T), DXXG, N(T)KXD, 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

C

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   1  ATTGAAAGGCTGATTCTTCTCTAAAGGATTTCTCTTCAGAGACAAAAAGAAACCACCCTGGACATGGCCTCAGAGATCCACATG
                                                                  M  A  S  E  I  H  M
  85  TCGGAACCCATGTGCCTCATTGAGAACACTGAGGCTCAACTAGTGATCAACCAGGAGGCTCTGAGGATCCTGTCTGCCATTACA
      S  E  P  M  C  L  I  E  N  T  E  A  Q  L  V  I  N  Q  E  A  L  R  I  L  S  A  I  T
 169  CAGCCTGTGGTGGTGGTGGCGATCGTGGGCCTCTACCGCACAGGCAAATCCTACCTGATGAACAAGCTAGCTGGGAAGAGGACA
      Q  P  V  V  V  V  A  I  V  G  L  Y  R  T  G  K  S  Y  L  M  N  K  L  A  G  K  R  T
 253  GGCTTCTCCCTGGGCTCCACTGTGCAGTCTCACACCAAGGGCATCTGGATGTGGTGTGTGCCTCACCCCAAGAAGGCAGGGCAA
      G  F  S  L  G  S  T  V  Q  S  H  T  K  G  I  W  M  W  C  V  P  H  P  K  K  A  G  Q
 337  ACCCTGGTTCTGCTTGACACTGAGGGCCTTGAAGATGTTGAGAAGGGTGACAACCAGAATGACTGCTGGATCTTTGCTTTGGCA
      T  L  V  L  L  D  T  E  G  L  E  D  V  E  K  G  D  N  Q  N  D  C  W  I  F  A  L  A
 421  GTCCTCCTCAGCAGCACCTTCATCTACAACAGCATAGGAACCATCAACCAGCAGGCCATGGACCAGCTGCACTATGTGACAGAA
      V  L  L  S  S  T  F  I  Y  N  S  I  G  T  I  N  Q  Q  A  M  D  Q  L  H  Y  V  T  E
 505  CTGACTGATCTCATCAAATCAAAGTCATCACCTGATCAGAGTGATGTAGACAACTCAGCTAACTTTGTGGGCTTTTTTCCTATC
      L  T  D  L  I  K  S  K  S  S  P  D  Q  S  D  V  D  N  S  A  N  F  V  G  F  F  P  I
 589  TTTGTGTGGACTCTGAGGGATTTCTCCCTGGATCTGGAATTTGATGGAGAATCCATCACTCCTGATGAGTACCTGGAGACTTCA
      F  V  W  T  L  R  D  F  S  L  D  L  E  F  D  G  E  S  I  T  P  D  E  Y  L  E  T  S
 673  CTGGCTCTGAGAAAAGGAACTGATGAGAACACTAAAAAATTTAATATGCCTCGCCTGTGTATCAGGAAGTTCTTCCCAAAGAGG
      L  A  L  R  K  G  T  D  E  N  T  K  K  F  N  M  P  R  L  C  I  R  K  F  F  P  K  R
 757  AAGTGCTTCATCTTTGACAGGCCTGGAGACAGGAAGCAACTTTCCAAACTAGAGTGGATACAGGAGGACCAGCTGAATAAAGAA
      K  C  F  I  F  D  R  P  G  D  R  K  Q  L  S  K  L  E  W  I  Q  E  D  Q  L  N  K  E
 841  TTTGTAGAACAAGTTGCAGAATTCACCTCATACATCTTCAGCTATTCTGGTGTCAAGACTCTATCTGGAGGCATCACAGTCAAT
      F  V  E  Q  V  A  E  F  T  S  Y  I  F  S  Y  S  G  V  K  T  L  S  G  G  I  T  V  N
 925  GGGCCACGTCTGAAAAGCCTGGTGCAGACCTATGTCAGTGCCATCTGCAGTGGAGAACTACCCTGTATGGAGAACGCAGTCCTG
      G  P  R  L  K  S  L  V  Q  T  Y  V  S  A  I  C  S  G  E  L  P  C  M  E  N  A  V  L
1009  ACTTTGGCCCAGATAGAGAACTCAGCAGCAGTGCAAAAGGCCATCACCTACTACGAAGAACAGATGAATCAGAAGATCCACATG
      T  L  A  Q  I  E  N  S  A  A  V  Q  K  A  I  T  Y  Y  E  E  Q  M  N  Q  K  I  H  M
1093  CCCACAGAAACCCTCCAGGAGCTCCTGGATCTGCACAGGACCTGTGAGAGGGAGGCCATTGAGGTCTTCATGAAGAATTCTTTC
      P  T  E  T  L  Q  E  L  L  D  L  H  R  T  C  E  R  E  A  I  E  V  F  M  K  N  S  F
1177  AAGGATGTAGACCAGAAGTTCCAGGAAGAATTAGGGGCCCAGCTGGAAGCCAAACGAGATGCCTTTGTTAAGAAGAACATGGAC
      K  D  V  D  Q  K  F  Q  E  E  L  G  A  Q  L  E  A  K  R  D  A  F  V  K  K  N  M  D
1261  ATGTCATCTGCTCATTGCTCAGACTTACTGGAGGGCCTCTTTGCTCATCTGGAAGAAGAAGTGAAGCAGGGGACATTTTATAAA
      M  S  S  A  H  C  S  D  L  L  E  G  L  F  A  H  L  E  E  E  V  K  Q  G  T  F  Y  K
1345  CCAGGAGGCTACTACCTTTTTCTTCAAAGGAAACAAGAGCTGGAGAAAAAGTATATCCAGACTCCTGGAAAGGGACTCCAGGCT
      P  G  G  Y  Y  L  F  L  Q  R  K  Q  E  L  E  K  K  Y  I  Q  T  P  G  K  G  L  Q  A
1429  GAAGTGATGCTGAGAAAATACTTTGAATCCAAGGAGGATTTGGCTGATACACTTCTAAAGATGGACCAGTCACTCACAGAAAAG
      E  V  M  L  R  K  Y  F  E  S  K  E  D  L  A  D  T  L  L  K  M  D  Q  S  L  T  E  K
1513  GAAAAGCAGATTGAAATGGAACGTATAAAAGCAGAAGCCGCAGAAGCAGCAAATAGAGCATTGGCAGAAATGCAAAAGAAGCAT
      E  K  Q  I  E  M  E  R  I  K  A  E  A  A  E  A  A  N  R  A  L  A  E  M  Q  K  K  H
1597  GAGATGCTGATGGAACAGAAGGAACAGAGTTATCAAGAGCACATGAAACAGCTGACTGAGAAGATGGAGCAGGAACGGAAAGAG
      E  M  L  M  E  Q  K  E  Q  S  Y  Q  E  H  M  K  Q  L  T  E  K  M  E  Q  E  R  K  E
1681  TTAATGGCAGAGCAACAAAGAATCATATCCCTTAAACTTCAGGAACAGGAAAGACTTCTCAAGCAAGGATTCCAGAATGAGAGC
      L  M  A  E  Q  Q  R  I  I  S  L  K  L  Q  E  Q  E  R  L  L  K  Q  G  F  Q  N  E  S
1765  TTGCAACTACGTCAAGAGATAGAGAAAATCAAGAACATGCCTCCACCTCGATCATGCACCATACTTTAAAATCTGAACAGACTA
      L  Q  L  R  Q  E  I  E  K  I  K  N  M  P  P  P  R  S  C  T  I  L  *
1849  AAGCTCTCTACCCTGTTTCCACTCATCAAGGAAAAAACTTCGGGGACAGCTTTGGATTGTGACACAGCATGGCATTGAATGAGA
1933  CTTGAAGACAATGACACTTTACTAGGAAGAAGGGAGAAATAAAGTTCACAAAGTGGAGTTTATATGTGAAAAAAATGTTTGGCT
2017  TATGAGGTGACTCAGTTCATGTTTGTGTTAGAAGTGAAAATTCATGTGATTTTTAATCGGGTCTTATATTTGATACTGCAAACA
2101  TGGACTCTGTAGACATACTAAATTGTATTGTTGGGATTTGTTTTTACAACACAGTGATAGGGCAGCTGTCTTTGGGTAGACATT
2185  TTGTATCACATACATCCCCTACTGGATATTCTTAGTTGTGTGCTCATAAGTAATTCAAAGATGTAGCTTGCTCTAAGCTGGGAT
2269  TGGCATGTTATAAACATGATAAAAATCTGAACATATCCATGGAACACAGCTCCTAGGGCCTCATGCTATCTGCAGACATTGGTT
2353  CCCAGGGTAGACAATGGGCAGTGCCCAATGTGGGAAGGCTCTGTGGTTGCTGGATGAGCAGAGTATTTCATTGGAGAGGAGAAG
2437  AAAATTCCCCTCCAGGCAGAATGCATAGGCGAGTGGGCGTTGCACCTCCCCCATTGAGAGAACTTCTTGTTTTCTTTCATGCTC
2521  CAGCCTACTGTGTTTCCTTTCTAATCTCTCTTTTTATTTTTCAAAAACACTTTCTCCTTCTAAGAGTTTTAGAATGACTTCTAC
2605  ACAGAATGGAGAACTCAAAATAAATTTCGTTCTATTCCTCTGAGGCTCATAATCACAGTGCCAACTCTTGTGAAACCAACCTTC
2689  ATTTTTATCTTTCTTTTGCTGCTATGACATGATTTGCATATCTCTGAAGATAAATGCCTTTTACAATGTGGCAGAAGCACTGTT
2773  TAAGTGAAAACTGTTAATAAAAATGATTAAGCTT(A)n
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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 sequence NKXD (nor the reversed motif DXKT or DXKN) 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 CAAX 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 CAAX 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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                                                          I                                                   II
hGBP-1  MASEIHMTGPMCLIENTNGRLMANPEALKILSAITQPMVVVAIVGLYRTGKSYLMNKLAGKKKGFSLGSTVQSHTKGIWMWCVPHPKKPGHILVLLDTEG   100
hGBP-2      QLA..NEP.D..K.Q.VV.............V......................N........K.................E.T.......      95
mGBP-1  .......SE........EAQ.VI.Q...R........V.....................RT.........................A.QT.......     100

hGBP-1  LGDVEKGDNQNDSWIFALAVLLSSTFVYNSIGTINQQAMDQLYYVTELTHRIRSKSSPDENENEVEDSADFVSFFPDFVWTLRDFSLDLEADGQPLTPDE   200
hGBP-2  ...I.....E.........I.........M...........H.....D..KAN...GN.--S.D..........A........T.E..V..E.I.A.D    193
mGBP-1  .E.........C.............I..............H.....DL.K......QS--D.DN..N..G...I.............F..ESI....    198

hGBP-1  YLTYSLKLKKGTSQKDETFNLPRLCIRKFFPKKKCFVFDRPVHRRKLAQLEKLQDEELDPEFVQQVADFCSYIFSNSKTKTLSGGIQVNGPRLESLVLTY   300
hGBP-2  ..EL....R...DK.SKS..D...........R......W.APKKY..H..Q.KE...N.D.IE...E.......H.NV.......A.............   293
mGBP-1  ..ET..A.R...DENTKK..M...........R...I....GD.KQ.SK..WI.EDQ.NK...E...E.T.....Y.GV.......T......K...Q..   298

hGBP-1  VNAISSGDLPCMENAVLALAQIENSAAVQKAIAHYEQQMGQKVQLPTESLQELLDLHRDSEREAIEVFIRSSFKDVDHLFQKELAAQLEKKRDDFCKQNQ   400
hGBP-2  ...........................E...........G......T...................MKN......QM..RK.G....AR........S   393
mGBP-1  .S..C..E.........T..............TY..E..N..IHM.............TC........MKN......QK..E..G....A...A.V.K.M   398

hGBP-1  EASSDRCSGLLQVIFSPLEEEVKAGIYSKPGGYRLFVQKLQDLKKKYYEEPRKGIQAEEILQTYLKSKESMTDAILQTDQTLTEKEKEIEVERVKAESAQ   500
hGBP-2  K....C.MA...D..G....D..Q.TF.........T....E..N...QV.......K.V.KK..E...DVA..L.....S.S....A.....I.....E   493
mGBP-1  DM..AH..D..EGL.AH......Q.TFY.....Y..L.RKQELE...IQT....L...VM.RK.FE...DLA.TL.KM..S......Q..M..I...A.E   498

hGBP-1  ASAKMLQEMQRKNEQMMEQKERSYQEHLKQLTEKMENDRVQLLKEQERTLALKLQEQEQLLKEGFQKESRIMKNEIQDLQTKMRRRK-A[CTIS]   592
hGBP-2  .AK...E.I.-...E......K.....V........R..A..MA...K.........R......EN..KRLQKD.W.I.MRSKSLEPI[.N.L]   586
mGBP-1  .ANRA.A...K.H.ML.....Q.....M........QE.KE.MA..Q.IIS.......R...Q...N..LQLRQ..EKIKNMPPP.--S[...L]   589
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FIG. 5. Comparison of hGBP-1, hGBP-2, and mGBP-1 protein sequences. A dot in the hGBP-2 or mGBP-1 sequence indicates that the corresponding amino acid is identical to that of hGBP-1. Nonidentical amino acids are spelled out. Dashes indicate gaps introduced to allow best fit. The underlined sequences correspond to the highly conserved GTP-binding consensus motifs I and II. The C-terminal CAAX motifs are boxed.

the 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). GBP-1 was recovered most efficently by GDP agarose, but GTP and GMP agarose were also potent affinity matrixes 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.

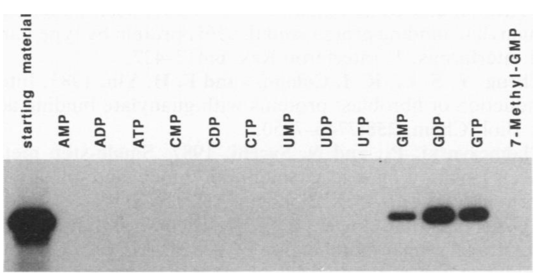

FIG. 6. Binding of mGBP-1 to GMP, GDP, and GTP but not other nucleotides. mGBP-1 cDNA was cloned downstream of the SP6 promoter, and capped RNA was synthesized and translated in vitro in the presence of [^{35}S]methionine. A sample of this material was analyzed by SDS-PAGE without further processing (starting material). Other samples were subjected to affinity chromatography. The matrixes were agarose beads loaded with the nucleotides indicated. After extensive washing of the beads, the bound protein was eluted and analyzed by SDS-PAGE.

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

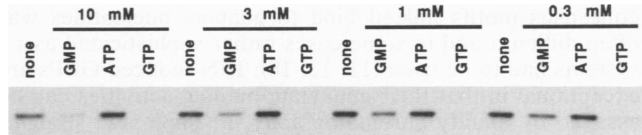

FIG. 7. Competition by GTP and GMP for binding of mGBP-1 to GMP agarose. GMP agarose affinity chromatography was performed as for Fig. 6 but in the presence of the indicated concentrations of GMP, ATP, or GTP.

abundant than hGBP-1 mRNA in IFN-treated FS-2 cells. The similarity of the deduced protein sequences suggests that hGBP-1 and hGBP-2 are isoforms 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 GXXXXGKS (T), DXXG, and N(T)KXD (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)KXD motif represents the guanine specificity region. Interestingly, the IFN-induced GBPs lacked the N(T)KXD 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 adenine, uracil, or cytosine nucleotides. Thus, IFN-induced GBPs exhibit a high degree of guanine nucleotide-binding specificity despite lacking the N(T)KXD 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)KXD 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 GXXXXGKS(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 genetics. Mouse strains that lack the ability to synthesize the IFN-induced 65-kDa GBP-1 protein were described, and classical genetics allowed the mapping of the *Gbp-1* gene to mouse chromosome 3 (27, 32). Our Northern studies with probes derived from cloned GBP-1 mRNA have now revealed the existence of multiple *Gbp-1*^{b} (noninducibility) alleles. GBP-1 mRNA was not detected in cells from strain C57BL/6, whereas small but significant amounts of this mRNA were found in the *Gbp-1*^{b} strain A2G. In contrast to the situation in *Gbp-1*^{a} mice, IFN failed to stimulate GBP-1 mRNA synthesis in A2G cells. The simplest interpretation of these results was that the *Gbp-1*^{b} allele of strain C57BL/6 was a severely crippled *Gbp-1* gene, whereas the *Gbp-1*^{b} allele of A2G mice represented a *Gbp-1* gene with a noninducible promoter. Genomic Southern blotting and sequencing experiments will be necessary to further evaluate these possibilities.

Earlier studies indicated that IFN-treated mouse cells synthesize several minor GBPs in addition to GBP-1 (32). The origin of the minor GBPs was unclear. Our data now suggest that these proteins were coded for by one or more genes related to but distinct from *Gbp-1*. It is conceivable that the minor GBPs can functionally complement the absence of GBP-1 in *Gbp-1*^{b} mouse strains. If so, this might explain why we were unable to detect a mutant phenotype associated with the *Gbp-1* mutations and why we found *Gbp-1*^{b} alleles at high frequencies in populations of inbred and wild mice (27).

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