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Two *cis*-DNA Elements Involved in Myeloid-Cell-Specific Expression and Gamma Interferon (IFN-γ) Activation of the Human High-Affinity Fcγ Receptor Gene: a Novel IFN Regulatory Mechanism

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The human high-affinity receptor for the constant region of immunoglobulin G (human FcγR1) is encoded by two mRNAs induced selectively by gamma interferon (IFN-γ) and expressed in cells of myeloid lineage. The *cis*-DNA element (GRR) previously found to confer IFN-γ responsiveness to this gene acts as an inducible enhancer and is the target of an IFN-γ-activated factor(s) (GIRE-BP) in cells of different origins. Although the GRR motif is not related to the DNA elements involved in the regulation of other IFN-stimulated genes, GIRE-BP binding depends on the IFN-γ-dependent activation of the 91-kDa protein known to be one of the factors of a transcriptional complex activated by IFN-α. Deletions of the FcγR1 promoter allowed us to identify a 25-bp element, downstream from the GRR motif, conferring cell-type-specific expression. This element, called MATE (myeloid activating transcription element), is the DNA target for constitutive factors forming two complexes, MATE-BP$_1$ and MATE-BP$_2$. In accordance with the functional analysis, MATE-BP binding activities were detected in extracts prepared from myeloid cell lines such as THP-1, HL-60, and U-937 but not in HeLa cell extracts. The MATE motif is present not only in the promoter of other Fc receptor genes but also in several promoters of genes whose expression is restricted to monocytic cells. Our results suggest that human FcγR1 gene expression in myeloid cells is initiated by the interaction of IFN-γ-activated factors with cell-type-specific factors through their binding to the GRR and MATE motifs.

Many of the cellular responses to the interferons (IFNs) require new RNA and protein synthesis (54). In particular, IFN-γ induces the expression of unique mRNAs and proteins in addition to those induced by IFN-α and IFN-β (23, 68). IFN-γ is the most potent lymphokine known for activating cells of the mononuclear phagocyte lineage by regulating the degree of differentiation. In these cells, IFN-γ induces or enhances numerous macrophage capabilities, such as tumoricidal activity (44), antigen presentation (46), phagocytosis, and cytokine production (16). While IFN-α/β and IFN-γ have antiviral properties, the maximal antiviral state in the promyelomonocytic U-937 cell line cannot be achieved by IFN-γ alone and requires the coaddition of tumor necrosis factor alpha (TNF-α) (70). These features suggest that in spite of existing qualitative similarities between the biological effects of IFN-α/β and IFN-γ, their activities are often distinguishable.

Although IFN-α/β and IFN-γ bind to different receptors (49), some genes are induced by both types of IFNs. Identification of IFN-responsive *cis*-DNA elements (7, 28, 36, 52, 65) has led to identification of IFN-activated DNA binding factors (8, 15, 17, 22, 35). Genes encoding the 2′,5′-oligo(A) synthetase (5), major histocompatibility complex (MHC) class I (57), and 9-27 protein (53) contain similar motifs named IRS or ISRE depending on the gene in which it was identified. This sequence, conferring sensitivity to both types of IFN, is also involved in the regulation of genes induced predominantly by IFN-α/β such as 6-16 (52), ISG15, and ISG54 genes (35).

Treatment of cells with IFN-γ followed by IFN-α results in an increase in transcription of IFN-stimulated genes (37). This synergistic effect is due to the use of an IFN-γ-induced *trans*-acting factor (ISGF3γ) forming in combination with three IFN-α-activated factors (ISGF3α), a multiprotein complex termed ISGF3 (24, 37). Recently (20), an additional sequence overlapping the ISRE, the IFN-γ-activated site (GAS), was found (19, 38) to be involved in the IFN-γ regulation of the gene encoding the guanylate-binding protein.

Nevertheless, neither the *cis* elements nor the binding factors characterized so far provide an explanation for the preferential regulation of certain genes by IFN-γ. The regulation of MHC class II genes by IFN-γ has been investigated by several laboratories, and elements, unrelated to ISRE, that determine their induction have been identified (4, 11, 43, 60, 64). Studies of the regulation by IFN-γ of genes such as those encoding IP-10 (39), the human invariant chain (3), and the monokine induced by IFN-γ (*mig*) (72) suggested that the *cis*-DNA elements identified as targets for the specific IFN-γ response show sequence identity with the ISRE. However, no functional assay has demonstrated the involvement of these ISRE-like motifs in gene regulation by IFN-γ.

Recently, a minimal sequence, GRR, was found to confer IFN-γ activation (6, 47) to a gene encoding the human high-affinity receptor for immunoglobulin G (human FcγR1/CD64) (2). While FcγRI gene expression induced by IFN-γ is restricted to cells of myeloid lineage, the GRR motif cloned in front of a reporter gene was sufficient to confer significant IFN-γ inducibility when transfected in HeLa cells (47).

In this article, we present data demonstrating that the GRR motif acts as an inducible enhancer and is involved in

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the formation of an IFN-γ-activated complex resulting from its association with a specific DNA binding protein(s) (GIRE-BP). Such a complex is detected as early as 5 min after the addition of IFN-γ, even in the presence of cycloheximide. GIRE-BP is observed not only in monocytic cell lines such as THP-1, HL-60, and U-937 but also in HeLa cells. We demonstrate that GRR is not related to motifs such as IRS/ISRE; GAS; or X, S, and Y boxes involved in the regulation of genes by both types of IFNs and in the regulation of MHC class II genes. However, the use of cellular mutants deficient in their IFN responses (30, 40, 67) and antibodies raised against subunits of ISGF3α (55) led us to conclude that at least some of the factors known to contribute to IFN-α-activated ISGF3 (24, 37) are also components of the IFN-γ-activated GIRE-BP complex.

We also characterize the region conferring cell-type-specific expression of the FcγR1 gene, which we named MATE for myeloid activating transcriptional element. In mobility shift assays, two specific complexes (MATE-BP_1 and MATE-BP_2) are identified when MATE is used as a probe. The formation of these complexes does not depend on IFN-γ treatment but requires ongoing protein synthesis. In contrast to the factors bound to the GRR motif, MATE-BP complexes are not detected in HeLa cells. In two promyelomonocytic cell lines, HL-60 and U-937, only one MATE-BP complex which migrates faster than the two complexes seen in the monocytic cell line THP-1 is detected. Interestingly, two overlapping regions within the MATE motif show homologies with promoter sequences of several genes expressed in myeloid cells.

MATERIALS AND METHODS

Cells. RAW 264.7, HeLa, HL-60, U-937, and THP-1 cells were obtained from the American Type Culture Collection. Murine monocyte-macrophage RAW 264.7 and human epithelial adenocarcinoma HeLa cell lines were grown as monolayer cultures in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum. Human promyelomonocytic HL-60 and U-937 and human monocytic THP-1 cell lines were grown in suspension in RPMI 1640 supplemented with 10% fetal calf serum.

IFNs and TNF. Recombinant human IFN-γ was a gift from Roussel Uclaf, and recombinant IFN-α was provided by Hoffmann-La Roche. Recombinant murine IFN and human TNF-α were produced by Genentech and provided by Boehringer Ingelhein.

Plasmid constructions. An *Eco*RI genomic fragment of 4.3 kb representing the 5′ exon and 592 bp of the 5′ flanking region of the human FcγR1 gene was used as DNA template to generate various fragments by the polymerase chain reaction technique.

To obtain simian virus 40 (SV40) chloramphenicol acetyltransferase (CAT) constructs [(206/45)-SV CAT) and (45/206)-SV CAT], oligonucleotide primers spanning the sequences from −206 to −186 and −63 to −45 (with numbering starting at the ATG) and having a *Bgl*II restriction site at each 5′ end were used. The resulting polymerase chain reaction fragment was then cloned in the unique *Bgl*II site of the reporter plasmid pCAT (Promega Corp., Madison, Wis.), which contains the bacterial CAT reporter gene downstream of the SV40 promoter.

Various other fragments of the human FcγR1 promoter were also cloned in the *Bam*HI restriction site of the vector pBRAMS CAT2 (29) containing the CAT gene under the control of the thymidine kinase (TK) promoter. Primers with a *Bam*HI restriction site and corresponding to the sequences from −165 to −141 and −158 to −128 were used to obtain constructs (165/128)-TK CAT, (128/165)-TK CAT, and (128/165)$_3$-TK CAT. The same primers with an *Eco*RV restriction site allowed us to clone the same region (165/128) at the *Eco*RV site located 1 kb from the TK promoter [(TKCAT-(165/128)]. FcγR1 genomic fragments extending from nucleotides −140 to −45 and −206 to −107 were generated by polymerase chain reaction with the following combination of primers having a *Bgl*II restriction site: −140 to −124 and −63 to −45 and −206 to −186 and −129 to −107. The amplified fragments were inserted into the *Bam*HI site of plasmid pBRAMS CAT2 in order to design the vectors (140/45)-TK CAT and (206/107)-TK CAT. The vectors (103/78)-TK CAT and (78/103)-TK CAT were built by cloning in the *Bam*HI site of pBRAMS CAT2 an oligomer of 25 bp obtained after annealing of the two strands corresponding to the sequence from −103 to −78 (numbering from the ATG): GGATTCAATTTCCTTCCTCTTTTCTAATTTGGATTC. After multimerization of the region from −165 to −128 at the *Bam*HI site of pGEM3Z (Promega Corp.), fragment *Sac*I-*Xba*I was cloned at the same restriction sites of the plasmids previously described to get vectors (GIRE)$_3$-TK CAT, (GIRE)$_3$-(140/45)-TK CAT, (GIRE)$_2$-(206/107)-TK CAT, (GIRE)$_3$-(103/78)-TK CAT, and (GIRE)$_3$-(78/103)-TK CAT. Fragment *Sac*I-*Xba*I containing a hexamer of the IRS sequence was excised from plasmid IRS-TK CAT (7) and inserted at the *Sac*I-*Xba*I sites of the vectors pBRAMS CAT2 and (103/78)-TK CAT.

Transfections. Cells were plated at 5×10^6 cells per 100-mm tissue culture dish 1 day before transfection. THP-1 and RAW 264.7 cells were transfected with 10 μg of DNA by the DEAE-dextran method (25): DNA was combined to 250 μg/ml of DEAE-dextran in a final volume of 1 ml of HBSS (5 mM KCl, 0.4 mM KH_2PO_4, 140 mM NaCl, 0.3 mM $Na_2HPO_4 \cdot 12H_2O$, 5.5 mM glucose, 5 mM EDTA). After 5 min at room temperature, the mixture (DNA/DEAE-dextran) was added to the cells previously washed with serum-free media and incubated for 90 min at 37°C and 5% CO_2. The cells were then shocked for 3 min in 10% dimethyl sulfoxide in HBSS and were washed with phosphate-buffered saline (PBS) once, and complete medium was added. Twenty-four hours later, cells were divided into two 60-mm tissue culture dishes. After 8 h, a cell layer of one dish from each transfection group was induced with 500 U of the cytokines per ml. The cells were then harvested 12 h after stimulation. Transfections of HeLa cells were performed by the calcium phosphate method as described previously (14).

Enzyme assays. Cells were harvested and CAT assays were performed as follows. Cells were washed three times with PBS. One milliliter of TEN buffer (40 mM Tris-HCl [pH 7.5], 1 mM EDTA, 15 mM NaCl) was added, and the cell solution was incubated for 5 min at room temperature. The cells were then scraped, and after 10 min of centrifugation at $3,000 \times g$ the cell pellet was resuspended in 150 μl of 0.2 M Tris-HCl (pH 8) and subjected to three freeze-thaw cycles. After heating at 60°C for 10 min, the extract was spun for 10 min at $14,000 \times g$. The CAT assay was performed with 50 μl of the supernatant of the extract with *n*-butyryl-coenzyme A and xylene extractions as described in the CAT enzyme assay system protocol of Promega Biotech. Activities were adjusted for differences in the protein concentrations of the extracts. β-Galactosidase activity in the cell extracts was assayed by the method described in reference 41, with *o*-nitrophenyl-β-D-galactopyranoside (Boehringer Mannheim Biochemicals) as the substrate.

Cell extracts. Cells were collected by centrifugation for 20 min at 3,000 × *g* and then washed twice with cold PBS. Extracts were prepared according to a modified protocol allowing rapid isolation of cytoplasmic and nuclear proteins (75). Cells were resuspended in 4 volumes of a solution containing 10 mM [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (pH 7.9), 0.4 M NaCl, 0.1 mM EDTA, 0.5 mM dithiothreitol, 5% (vol/vol) glycerol, 0.5 mM phenylmethylsulfonyl fluoride, 0.1 μg of leupeptin per ml, 4 μg of aprotinin per ml, 1 μg of chymostatin per ml, 1.5 μg of pepstatin per ml, and 2 μg of antipain per ml. Resuspended cells were frozen in liquid nitrogen once and after thawing were homogenized by pipetting and then centrifuged for 5 min at 100,000 × *g*. Supernatants were then aliquoted and kept at −70°C. Cytoplasmic extracts were prepared with the same lysis buffer except that 0.14 M NaCl was used. After being frozen, the cell preparations were pelleted by centrifugation for 5 min at 10,000 × *g*. The supernatant was considered to be the cytoplasmic fraction. The pellet was then resuspended in 0.4 M NaCl and was treated as described for the whole cell extracts. After centrifugation at 100,000 × *g*, the resulting supernatant corresponded to the nuclear fraction.

Electrophoretic mobility shift assay. After preincubation of whole cell extracts (10 μg of proteins in 4 μl of 5× binding buffer containing 20% Ficoll, 100 mM HEPES [pH 7.5], 10 mM dithithreitol, 300 mM KCl, 0.05% Nonidet P-40, and 500 μg of bovine serum albumin per ml) with 10 μg of sonicated salmon sperm and eventually competitors, 5,000 to 10,000 cpm (Cerenkov) of ^{32}P-labelled probe was added and incubation at room temperature was allowed to proceed for 30 min in a final volume of 20 μl of mixture. Incubation was followed by gel electrophoresis. Oligonucleotides or DNA fragments were labelled with [γ-^{32}P]ATP (5,000 Ci/mmol; Amersham) with T4 polynucleotide kinase (Pharmacia). The nuclear factor 1 (NF1) binding site was provided in the footprinting kit from Pharmacia. All the samples were loaded on a 4% native polyacrylamide gel (44:1 acrylamide/bisacrylamide). The gel was run at 150 V in 0.5× TBE buffer (25 mM Tris, 25 μM boric acid, 1.15 μM EDTA). After drying, gels were exposed to Kodak XAR-5 films at −70°C.

The sequences of the oligonucleotides used for competition were as follows: for the IRS (15), TGAGGAAACGAA ACCA; for the Y and X/S boxes of the murine Eα gene (21), ATTTTTCTGATTGGTTAAAAGT and TAGGACCTGGTT GCAAGGAACCCTTTCCTAGCAACAGATGTGTC, respectively (homology with the human DRα gene is underlined); for the IFN-γ-activated site (GAS), (20) GATCCGTCAGTTTC ATATTACTCTAAATCCA.

RESULTS

Human FcγRI gene regulation in human or murine myeloid cells depends on an IFN-γ responsiveness enhancer. The *cis*-DNA element conferring IFN-γ responsiveness to the promoter of the FcγR1 gene was recently shown (47) to be localized between positions −168 and −132, with numbering starting at the ATG (Fig. 1). This sequence was named GRR, for IFN-γ response region (47). A fragment including GRR was cloned in front of a reporter gene encoding CAT under the control of the SV40 and TK promoters. CAT constructs (206/45)-SV CAT and (165/128)-TK CAT responded to IFN-γ when transfected into human THP-1 and murine RAW 264.7 monocytic cell lines (Fig. 2). Cloning of this motif in an inverted orientation [(45/206)-SV CAT, (128/165)-TK CAT] or at 1 kb 5′ to the TK promoter [TK CAT-(165/128)] did not affect IFN-γ-induced CAT activity (Fig. 2). In addition, a trimer of the region from −165 to −128 linked to the TK CAT vector [(GIRE)$_3$-TK CAT] led to an increased IFN-γ response compared with the activity of the (165/128)-TK CAT construct (Fig. 2). Therefore, these results suggested that GRR was acting as an inducible enhancer.

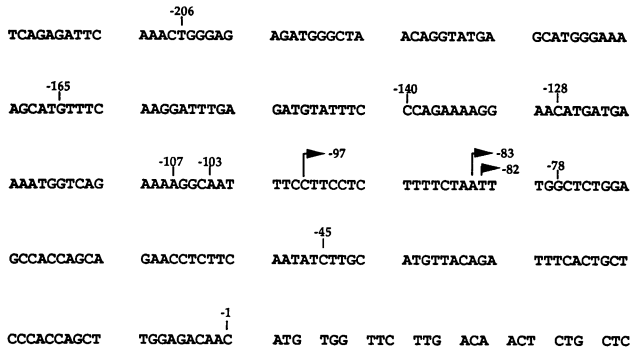

FIG. 1. Nucleotide sequence of the 5′ end of the FcγR1 gene. Sequencing was done by the dideoxy sequencing method. Numbering is from the adenosine (+1) of the ATG initiator codon. The previously identified RNA starts (6) are shown by the arrows.

Evidence for different IFN-γ regulation mechanisms was supported by the distinct responsiveness of FcγR1 and 2′,5′-oligo(A) synthetase genes in cells of monocytic lineage

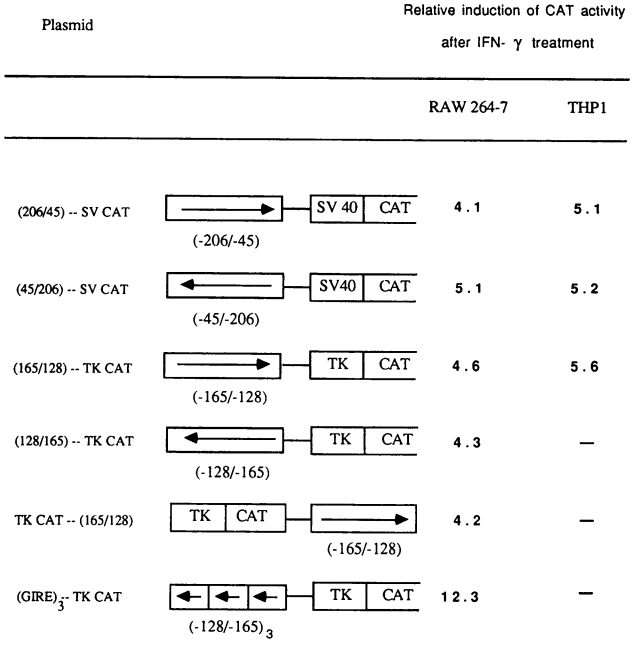

FIG. 2. IFN-γ-induced FcγR1 gene expression depends on the presence of an element characterized as an inducible enhancer. THP-1 cells and RAW 264.7 cells were transfected with various constructs as detailed in Materials and Methods. At least five independent experiments were performed with each construct. The standard deviations were 15 to 30% of the mean values. An SV40 β-galactosidase chimeric gene was cotransfected, and this activity was used as an internal control to monitor the efficiency of transfection. Results are expressed as the induction ratio derived from the mean counts per microgram of proteins correlated to the same β-galactosidase value found in induced versus uninduced extracts.

TABLE 1. Differential effect of cytokines on the expression of CAT vectors carrying the 5′ flanking sequence of the FcγR1 (6) gene and the IRS (7) in THP-1 cells[a]

Construct	Expression with:			
	IFN-α	IFN-γ	TNF-α	IFN-γ + TNF-α
FcγR1 CAT	0.48	5.60	0.98	5.30
(165/128)-TK CAT	0.75	5.30	0.85	4.80
pGEM CATE	14.72	0.38	0.55	2.00
IRS-TK CAT	5.30	0.37	0.39	2.50

[a] THP-1 cells were transfected with different CAT constructs (described in the text) and treated after transfection with 500 U of IFN-α, IFN-γ, and TNF-α. The results, determined from at least three independent experiments, are expressed as described in the legend to Fig. 2.

after treatment with different cytokines. It has been reported (70) that 2′,5′-oligo(A) synthetase mRNAs were poorly expressed in monocytic cell lines after treatment with IFN-γ or TNF-α alone, whereas combined treatment with these two cytokines strongly induced their expression. In order to test whether such a requirement was related to a transcriptional event and whether the IRS was the target of the synergistic effect observed, two CAT constructs were used (Table 1). The CAT gene was either under the control of the 2′,5′-oligo(A) synthetase promoter (pGEM CATE) (7) or under the control of the TK promoter cloned 3′ to a hexamer of the IRS motif (IRS-TK CAT) (7). The responsiveness of both constructs to cytokine treatment was compared with the activity of either $(GIRE)_3$-TK CAT or a vector (FcγR1 CAT) with the CAT gene controlled by the 5′ flanking region (−799 to −45) of the human FcγR1 gene (6). Although these last two constructs responded to IFN-γ alone in THP-1 cells (Table 1), an induced CAT activity with the pGEM CATE or the IRS-TK CAT vectors was seen only after IFN-α treatment or after coinduction with IFN-γ and TNF-α (Table 1). These results were in agreement with previous observations showing the requirement of both cytokines for induction of the 2′,5′-oligo(A) synthetase activity in the promyelomonocytic cell line U-937 (70). Therefore, the finding that GRR and IRS minimal sequences responded differently to IFN-γ in the myeloid cell line THP-1 suggested that these *cis*-DNA elements were distinct from each other.

GRR is a target sequence for specific IFN-γ-activated DNA binding proteins. To identify the *trans*-acting factors interacting with the GRR motif, band shift experiments (Fig. 3) were performed with the region from −165 to −128 as a probe (GIRE probe). Such experiments allowed us to identify a specific complex (GIRE-BP) present only in IFN-γ-treated cell extracts prepared from THP-1 (Fig. 3a) or HeLa (Fig. 3b) cells. Sequence identities among the GRR motif and the other consensus sequences known to confer IFN responsiveness have been reported previously (47). In order to test the significance of such homologies, competition experiments were performed with different motifs such as IRS (7); GAS (19); and the X, S, and Y boxes of the Eα MHC class II promoter (33). GIRE-BP was not detected after preincubation of the cell extract with an excess (10-fold) of the unlabelled fragment from −165 to −128 (Fig. 3a and b, lanes C_o), whereas no significant competition was observed with a large excess (100-fold molar) of competitors such as IRS (Fig. 3a and b, lanes C_1, and Fig. 4); GAS (Fig. 4); and the X, S (Fig. 3a and b, lanes C_2), and Y boxes (Fig. 3a and b, lanes C_3).

ISGF3α and ISGF3γ subunits are involved in the formation of the GIRE-BP complex. In THP-1 cells (data not shown) or

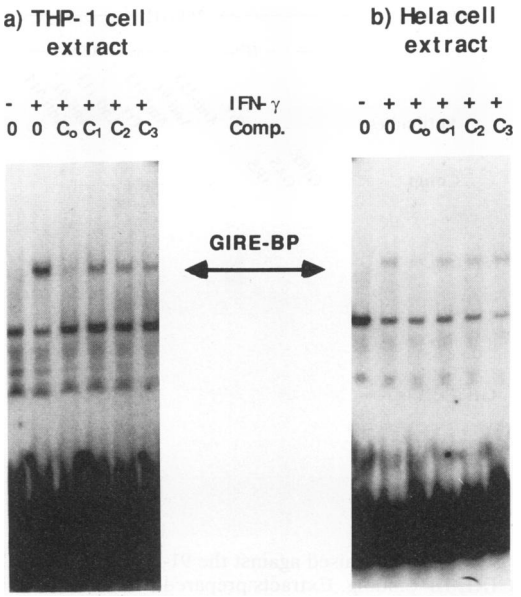

FIG. 3. IFN-γ induction of the binding of a specific complex to the GRR motif is not dependent on the cell type. Gel electrophoresis mobility shift assays were performed as described in Materials and Methods. Ten micrograms of untreated (−) and IFN-γ-treated (+) THP-1 (a) and HeLa (b) whole cell extracts were mixed with a ^{32}P-labelled DNA fragment from −165 to −128 (GIRE probe) after preincubation with the following DNA sequences (see Materials and Methods) as competitors: unlabelled sequence from −165 to −128 used in a 10-fold molar excess (C_o); 100-fold molar excesses of IRS and X/S and Y boxes (C_1, C_2, and C_3, respectively).

in a 2fTGH fibrosarcoma cell line derivative (48), a complex with the same electrophoretic mobility as the GIRE-BP was detected after IFN-α treatment (Fig. 4). Although the IFN-α-activated GIRE-BP complex was much less abundant than in cells treated with IFN-γ, the sensitivity to the different

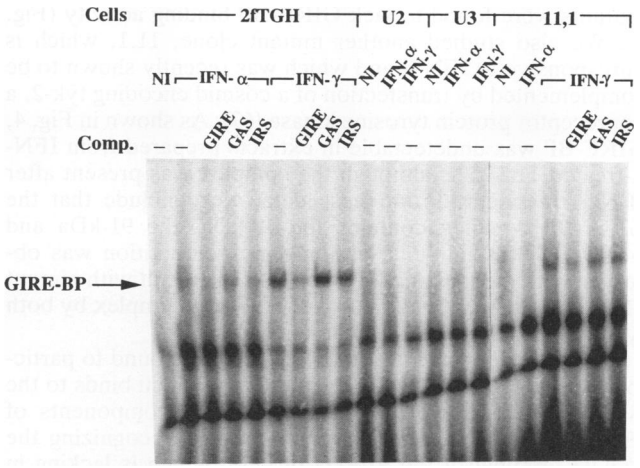

FIG. 4. GIRE-BP binding activity depends on factors forming the ISGF3 complex. Nuclear extracts were prepared from the different cellular mutants (described in the text) and from parental cells (2fTGH). Cells were treated with 500 U of human IFN-γ or IFN-α for 30 min. A 60-fold molar excess of competitor sequences such as the GIRE fragment (−165/−128) including the GRR motif, GAS, or IRS were incubated with extracts of IFN-treated cells before the addition of the GIRE probe.

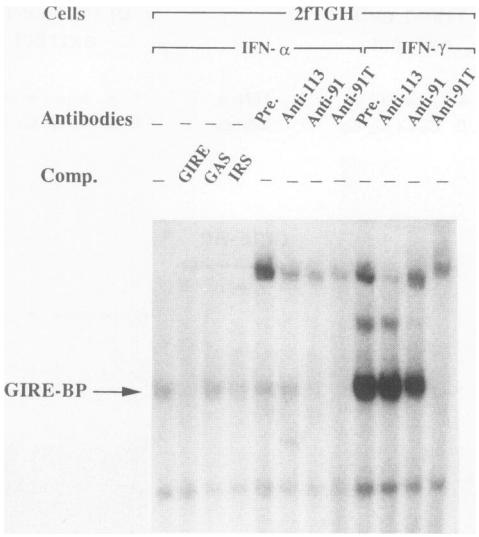

FIG. 5. Antibodies raised against the 91-kDa subunit of ISGF3α inhibit GIRE-BP binding. Extracts prepared from 2fTGH cells were incubated for 30 min at 4°C in the presence of sera (55) at a 1/150 final dilution. Competitors (Comp.) and the GIRE probe were added as described in the legend to Fig. 4.

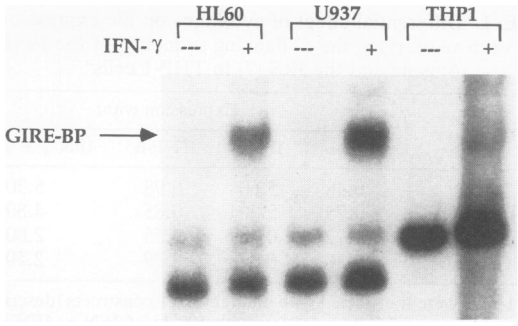

FIG. 6. GIRE-BP binding activity is present in different cell lines of myeloid origin. Whole cellular extracts from untreated (---) or IFN-γ-treated (+) HL-60, U-937, and THP-1 cells were incubated in the presence of the probe −165/−128 and were assayed for the presence of GIRE-BP binding activity by gel shift analysis.

competitors tested was similar (Fig. 4). The biological significance of such an observation is still unclear since neither the FcγR1 gene nor the $(GIRE)_3$-TK CAT construct (Table 1) can be induced with IFN-α.

To test the possibility of whether the GIRE-BP complex could be formed by the association of factors involved in the IFN-α response, such as ISGF3α and ISGF3γ, different IFN response mutants isolated from chemically mutagenized 2fTGH cells (30, 40) were used (Fig. 4). Mutant U2 (30), in which the ISGF3γ (Eγ) subunit is truncated, is unresponsive to IFN-α/β and partially defective in its response to IFN-γ. Mutant U3 (40), which lacks the 91- and 84-kDa subunits of ISGF3α (41a), is insensitive to both types of IFN. Extracts prepared from either IFN-α- or IFN-γ-treated U2 or U3 mutants were found to lack GIRE-BP binding activity (Fig. 4). We also studied another mutant clone, 11,1, which is unresponsive to IFN-α and which was recently shown to be complemented by transfection of a cosmid encoding tyk-2, a nonreceptor protein tyrosine kinase (67). As shown in Fig. 4, GIRE-BP was undetectable in extracts prepared from IFN-α-treated 11,1 cells although the complex was present after IFN-γ treatment. From these data, we conclude that the GIRE-BP complex contains the 48-kDa, the 91-kDa and 84-kDa ISGF3 subunits, although no competition was observed with the IRS and GAS motifs. The result with mutant 11,1 shows that activation of the GIRE-BP complex by both types of IFN depends on different pathways.

In order to determine among the proteins found to participate in the GIRE-BP complex the factor which binds to the GRR motif, antibodies raised against the components of ISGF3α (55) were used (Fig. 5). Anti-91T recognizing the COOH terminal of the 91-kDa protein, which is lacking in the 84-kDa protein (55), blocked the formation of the GIRE-BP complex (Fig. 5). Although anti-91T recognizing an amino acid sequence of the 91-kDa factor shared with the 84-kDa protein (55) partially affected GIRE-BP binding activity, no effect was seen with an antibody raised against the 113-kDa protein (Fig. 5). Moreover, the same reactivities to antibodies were obtained with extracts prepared from cells treated with IFN-α or IFN-γ (Fig. 5). These results strongly suggest that treatment with both types of IFN lead to the activation of a complex in which the 91-kDa protein is involved.

It has been shown that the requirement of protein synthesis for IFN-γ transcriptional induction depends on the gene studied (19, 65). Preincubation of THP-1 cells for 3 h with cycloheximide before the addition of IFN-γ did not affect the appearance of the complex (data not shown). GIRE-BP was detected after 5 min of IFN-γ treatment, and its level remained stable for at least 2 h (data not shown), suggesting that IFN-γ-induced activity was dependent on rapid IFN-γ activation of preexisting specific *trans*-acting factors. In two other promyelomonocytic cell lines, HL-60 and U-937, the GIRE-BP binding activity was present and was even stronger in U-937 cell extracts (Fig. 6).

A distinct *cis*-DNA element confers myeloid cell expression. It has been shown previously that cloning of the region from −169 to −141 (GRR) in front of the TK promoter led to a construct whose expression after IFN-γ induction was not restricted to myeloid cell lines (47).

To further characterize the sequence conferring myeloid-cell-specific gene activation of the FcγR1 gene, various fragments of the promoter were cloned in TK CAT vectors. While the $(GIRE)_3$-TK CAT vector was inducible in HeLa cells (Fig. 7), the region from −140 to −45 [$(GIRE)_3$-(140/45)-TK CAT] abolished the induced expression in these cells but did not affect the response in the monocytic cell line RAW 264.7 (Fig. 7). To define the 5′ border of the domain conferring the cellular specificity, the region from −206 to −107 (Fig. 1) containing the GRR motif was cloned in a $(GIRE)_2$-TK CAT construct (Fig. 7). Insertion of this sequence in the $(GIRE)_2$-(206/107)-TK CAT vector did not change the induced CAT expression (Fig. 7), suggesting that cell specificity was dependent on an element located downstream of position −107. Conclusive evidence was obtained with the construct $(GIRE)_3$-(103/78)-TK CAT containing a shorter sequence spanning the region from −103 to −78 (Fig. 7). CAT activity from this construct was again dependent on the type of cell transfected (Fig. 7). The possibility that the presence of the start sites of transcription could affect the expression of the CAT gene was ruled out by the similar results obtained with the construct $(GIRE)_3$-(78/103)-TK CAT (Fig. 7) containing the region from −103 to −78 cloned in an inverted orientation. However, it has to be noted that with these constructs, the induced CAT activity if reduced

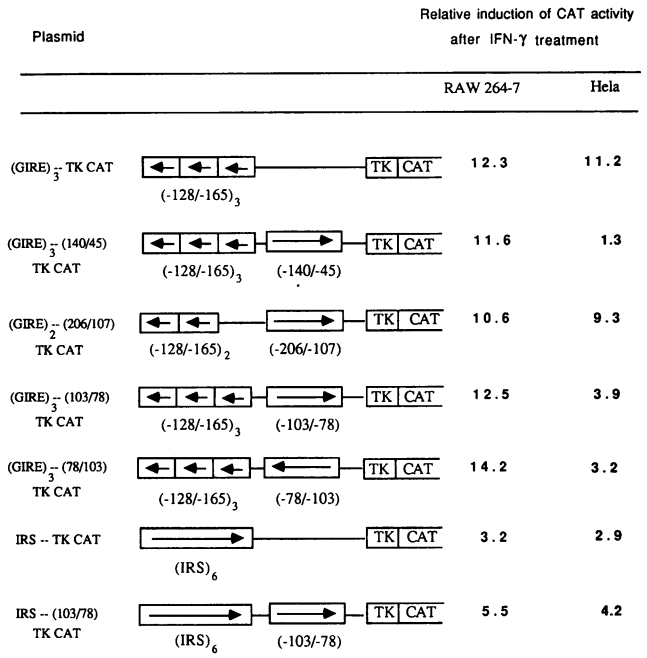

FIG. 7. Cell-type-specific expression of the human FcγR1 gene depends on a 25-bp fragment located downstream from the GRR motif. Results are the means of at least five independent experiments. RAW 264.7 cells, when transfected with the IRS-TK CAT construct, were coinduced with 500 U of IFN-γ and TNF-α per ml, while the other CAT activities shown were derived from cells treated with 500 U of IFN-γ per ml.

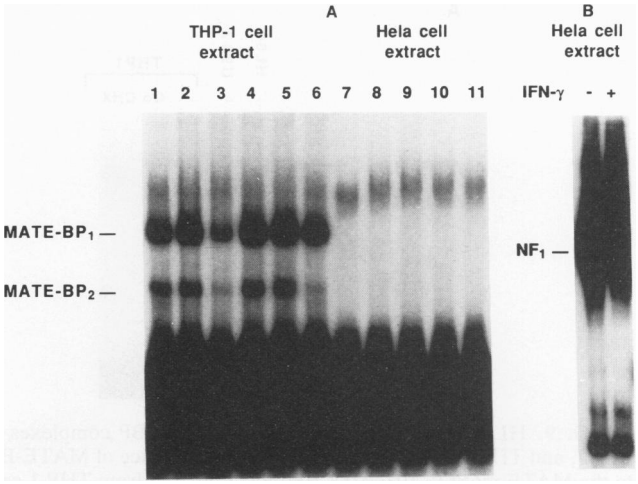

FIG. 8. Cell-type-specific expression of the FcγR1 gene correlates with the presence of two cell-type-specific complexes. (A) The ^{32}P-labelled DNA fragment corresponding to the region from −140 to −45 was added to whole cell extracts prepared from THP-1 cells (lanes 1 to 6) and from HeLa cells (lanes 7 to 11). Cells were not treated (lanes 1 and 7), or were treated with IFN-γ (lanes 2 to 6 and 8 to 11) and preincubated in the presence of a 10-fold molar excess of specific competitor (lanes 3 and 9) or a 50-fold molar excess of nonspecific competitors (IRS, lanes 4 and 10; NFκB, lanes 5 and 11). Lane 6 represents a cellular extract preincubated for 3 h with 10 μg of cycloheximide per ml before the addition of IFN-γ. (B) Control experiment. A sequence containing the NF1 binding motif (see Materials and Methods) was incubated with the same untreated (−) or IFN-γ-treated (+) HeLa cell extracts.

(fourfold) was not completely abolished in HeLa cells, in contrast to the repressed expression (ninefold) derived from the (GIRE)$_3$-(140/45)-TK CAT vector (Fig. 7). Nevertheless, these data indicated that the presence of the sequence from −103 to −78 was crucial for the restricted expression of the human FcγR1 gene in monocytic cells. Thus, we have named this 25-bp element involved in cell-type-specific expression of the FcγR1 gene promoter MATE, for myeloid activating transcription element. Interestingly, the MATE motif was not able to reduce the CAT expression of the IRS-TK CAT in HeLa cells as shown with the unchanged induced expression deduced from the IRS-(103/78)-TK CAT construct (Fig. 7). This specificity suggested that the MATE motif was acting only in the proximity of the GRR motif and strengthens the differences between GRR and IRS sequences.

FcγR1 gene expression in myeloid cells depends on cell-type-specific *trans*-acting factors. We tried to correlate the differential expression observed in CAT assays between myeloid and nonmyeloid cell lines with the presence of cell-type-specific *trans*-acting factors. Band shift experiments (Fig. 8) were performed with a fragment from −142 to −45 (Fig. 1), including the downstream part of GRR and the MATE motif as a probe. Incubation of this probe with untreated (Fig. 8A, lane 1) and IFN-γ-treated (Fig. 8A, lane 2) THP-1 extracts revealed two specific complexes, MATE-BP$_1$ and MATE-BP$_2$. Both complexes were observed in uninduced and induced cell extracts, confirming that the region from −142 to −45 was not involved in the induction but rather in the constitutive expression of the FcγR1 gene. Formation of the two complexes was significantly reduced in extracts prepared from cells treated with cycloheximide (Fig. 8A, lane 6). In the same experiment, the probe −142/−45 (Fig. 8A, lanes 7 to 11) or a probe presenting an affinity for a factor such as NF1 as a control (Fig. 8B) was incubated with cell extracts prepared from HeLa cells. While NF1 was binding at near completion to its specific target (Fig. 8B), no complexes were detected with the probe −142/−45 in uninduced or induced HeLa cell extracts (Fig. 8A, lanes 7 to 11). In order to confirm that the binding activity was dependent on the presence of the minimal sequence identified by the CAT analysis, an oligomer corresponding to the region from −103 to −78 was used as a probe for further mobility band shift assays. As seen in Fig. 9A, again two specific complexes, MATE-BP$_1$, and MATE-BP$_2$, were detected after incubation of the probe with whole cell extract prepared from IFN-γ-treated (Fig. 9A) and untreated THP-1 cells (data not shown). However, these complexes, which were both affected by cycloheximide treatment (Fig. 9A), migrated slightly differently from the previous complexes obtained with the probe −142/−45. Interestingly, in two other promyelomonocytic cell lines, HL-60 and U-937, only one complex (MATE-BP$_3$) migrating faster than the MATE-BP complexes identified in THP-1 cells was detected (Fig. 9B). This complex was specific, since a fivefold excess of the corresponding motifs −103/−78 and −142/−45 (Fig. 9B) was able to reduce the binding activity. Moreover, the −103/−78 probe was not able to form any of the identified complexes when incubated with HeLa cellular extracts (Fig. 9B). Therefore, the correlation observed between CAT assays and band shift experiments strongly suggested that the restricted expression of the FcγR1 gene in cells of the monocytic lineage was dependent on the binding of constitutive cell specific factors to the MATE motif.

Potential sequence identity within the MATE motif. Besides

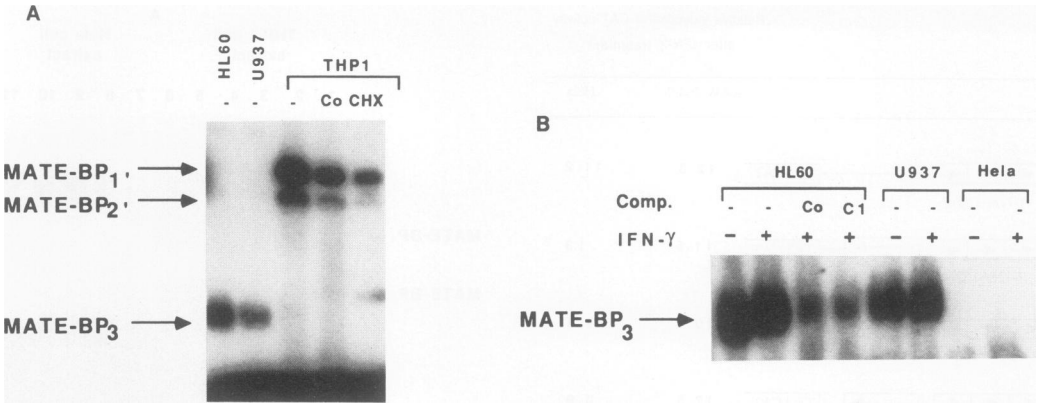

FIG. 9. HL-60 and U-937 cells present MATE-BP complexes different from those observed in THP-1 cells. (A) Extracts from HL-60, U-937, and THP-1 cells were assayed for the presence of MATE-BP complex formation by gel shift analysis with an oligomer corresponding to the MATE motif (−103/−78). Extracts prepared from THP-1 cells were treated with cycloheximide (CHX) as described for Fig. 8A, lane 6. A fivefold molar excess of homologous competitor DNA (Co) was included in the binding reaction to demonstrate specificity. (B) HL-60, U-937, and HeLa cell extracts were incubated in the presence of the same probe used for panel A. Specific binding was checked by preincubating the HL-60 extract with a fivefold molar excess of either the unlabelled fragment from −103 to −78 (Co) or the fragment from −140 to −45 (C1).

the sequence conservation between the human and murine FcγR1 promoters (47, 69), it has been reported that the GRR motif contains some similarities to other elements involved in the IFN-γ response such as γ-IRE and X and H boxes (47). The potential homologies found with the X and H (also known as S or W) boxes are not clear and are not supported by our competition experiments (Fig. 3, lanes C_2 and C_3). Although the sequence identity between γ-IRE and the GRR motif will have to be investigated functionally, our data (Table 1 and Fig. 3, lanes C_1) suggest that the IRS/ISRE and γ-IRE sequences (74) are unrelated.

Within the MATE region, a 14-bp sequence located on the FcγR1 gene (Fig. 10A) at positions −100 to −87 was conserved in the promoter of the murine αFcγR gene (10). A similar sequence was found (Fig. 10B) in the promoters of the *mig* gene (72) and the cathepsin G gene (27), whose expressions are also restricted in cells of monocytic lineage and in neutrophils. Interestingly, the sequence of the MATE motif from −94 to −85, which contains a pyrimidine-rich sequence, was found (Fig. 10B) in several myeloid-cell-specific promoters such as c-*fes* (1), CD11b (45), gp91-phox (56), and human neutrophil elastase (58). In addition, the sequence from −96 to −91 corresponded in an inverted orientation (Fig. 10B) to an element defined as the PU box (5′-GAGGAA-3′) (50), which binds a transcriptional activator (PU.1) expressed in macrophages and B cells (32). From these homologies, we were able to deduce two overlapping consensus sequences, MATE-c1 and MATE-c2 (Fig. 10C), which could be involved in the activation of a large number of genes expressed in a cell-type-specific manner. Such overlapping sequences are likely to be the target of factors forming the MATE-BP complexes.

DISCUSSION

In this article, we demonstrate that the IFN-γ response region (GRR), previously identified in the human FcγR1 promoter (6, 47), acts as an inducible enhancer. This sequence, which allows an IFN-γ response even in cells of nonmyeloid origin, is the target for an IFN-γ-activated factor(s) (GIRE-BP). Following IFN-γ addition, the activation of this complex is observed in nuclear extracts prepared from different cell types within minutes and does not require protein synthesis.

We next addressed the question of whether the GRR motif contains similarities to the sequences already defined as targets of IFN regulation. In gel mobility shift assays, no competition is observed with the IRS and GAS elements identified in the promoters of IFN-stimulated genes or the X/S and Y boxes involved in IFN-γ regulation of MHC class II genes. Different mechanisms leading at least to IFN-γ induction of FcγR1 and MHC class II genes are supported by experiments performed with mutants defective in their responses to IFN-γ for MHC class II gene expression (39a). These mutants, belonging to different complementation groups, exhibit a GIRE-BP binding activity similar to that of the parental cells (data not shown).

However, the absence of competitions between GRR and the other motifs did not exclude the possibility that common factors, such as the proteins involved in ISGF3 (24, 37), could recognize different sequences. The mechanism by which IRS/ISRE triggers IFN-α regulation has been well documented (55). Upon IFN-α treatment, three cytoplasmic proteins of 113, 91, and 84 kDa, collectively termed ISGF3α, are translocated to the nucleus with another protein of 48 kDa, ISGF3γ (31). This latter protein, associated with ISGF3α, has been shown to bind to the ISRE motif and to be induced with IFN-γ (31, 37). Synergy between IFN-α and IFN-γ (61) for biological functions and induction of IFN-stimulated genes have been observed with HeLa cells. Moreover, a set of IFN-stimulated genes (31, 34) has shown higher-than-normal induction following combined treatment with IFN-γ and IFN-α/β due to the induction of ISGF3γ (37). Therefore, these observations suggested that both IFN pathways use the same components.

Although neither the FcγR1 gene nor a construct such as the $(GIRE)_3$-TK CAT is induced with IFN-α (Table 1), GIRE-BP binding activity is also observed in extracts prepared from IFN-α-treated cells such as 2fTGH (Fig. 4) or THP-1 cells (data not shown).

The use of different mutants defective in their IFN responses (Fig. 4) allows us to identify some of the factors involved in the GIRE-BP complexes activated by both types of IFN. At least the following two subunits, the 48-kDa

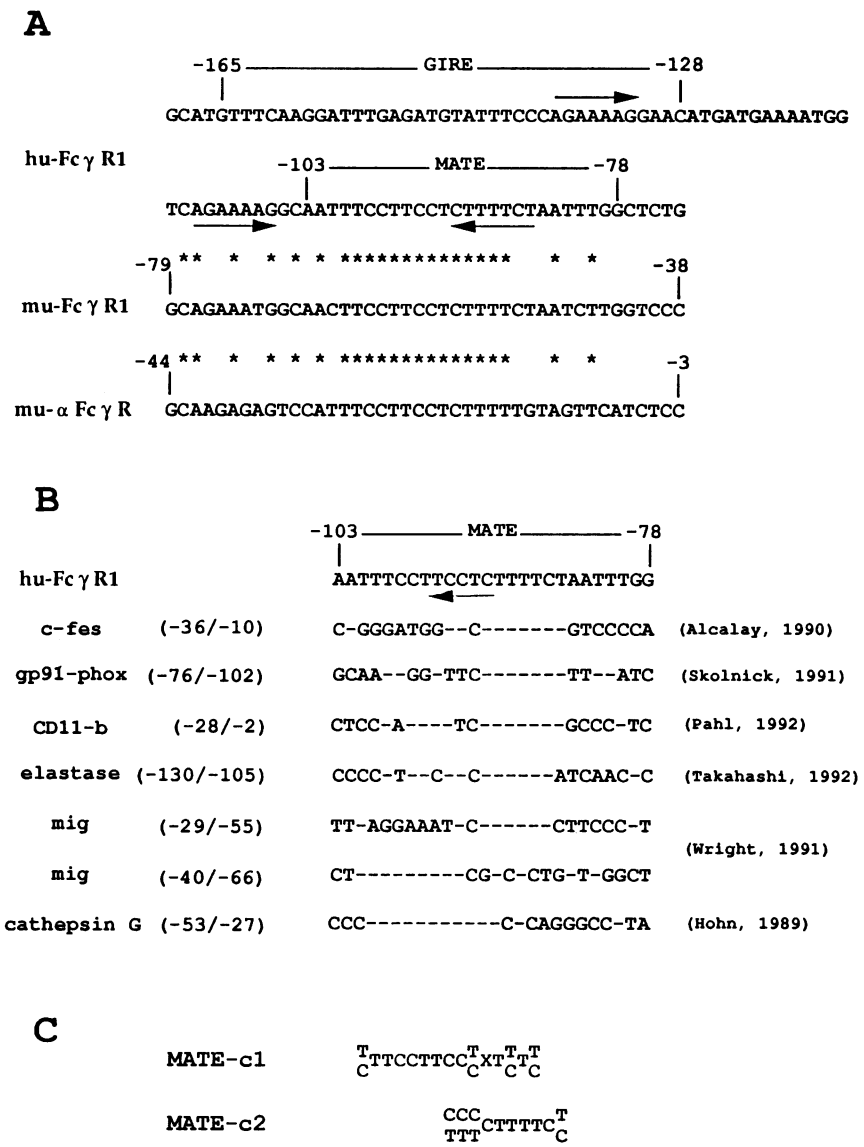

FIG. 10. Homology within the MATE motif. (A) Conserved nucleotides according to the maximal homology alignment are noted by asterisks, and the three AGAAAAG repeats are marked by black arrows. For the three Fc receptor sequences, numbering starts at the ATG. (B) Dashed lines indicate that the same nucleotide is present in the human FcγR1 (hu-FcγR1) promoter sequence. For the *mig*, elastase, gp91-phox, CD11b, and cathepsin G promoter sequences, the numbering is relative to the distance from the transcription start site, while for the c-*fes* sequence containing a first CAP site in the following upstream sequence of exon 2, the numbering is according to the position of the first ATG in this exon. The putative PU box is underlined by a black arrow. (C) Two overlapping consensus sequences are derived from the maximal alignment between the Fc promoter sequences and either the *mig* and cathepsin G genes (MATE-c1) or with *mig*, elastase, gp91-phox, CD11b, and c-*fes* genes (MATE-c2).

subunit (ISGF3γ) and the 91- or 84-kDa subunit from ISGF3α, seem to be required to form an IFN-activated GIRE-BP complex. However, our results with the non-IFN-α-responsive mutant 11,1, which is defective in the expression of the tyrosine kinase tyk-2 (67), suggest that IFN-dependent phosphorylation is an event distinguishing IFN-α and IFN-γ activation.

It has been shown recently (55) that whereas IFN-α binding to fibroblast cells leads to immediate phosphorylation of the 113-, 91-, 84-kDa proteins, IFN-γ binding to fibroblasts leads to phosphorylation of only the 91-kDa protein. These observations and the absence of GIRE-BP activity when antibodies raised against the 91-kDa protein are used (Fig. 5), suggest that this protein contacts the GRR DNA motif.

Although both types of IFN lead to the formation of similar GIRE-BPs, the phosphorylated 91-kDa protein will interact differently with the 48-kDa protein and eventually with other factors depending on the IFN used. Indeed, a different affinity of GIRE-BP for the GRR motif is suggested by the different intensity of the GIRE-BP binding activities observed after IFN-γ and IFN-α induction (Fig. 4).

The restricted expression of the FcγR1 gene in myeloid cells and the absence of CAT activity in HeLa cells with vectors containing the GRR motif followed by its downstream sequence lead us to define the region conferring cell

type specificity. Transfections of HeLa and RAW 264.7 or THP-1 cells with different constructs allow us to identify a sequence of 25 bp (MATE) which confers cell-type-specific expression (Fig. 7). This sequence is the target of two distinct complexes (MATE-BP) detected in THP-1 but not in HeLa cells (Fig. 8). Neither complex is regulated by IFN-γ, and both are sensitive to cycloheximide. Only one complex, migrating faster than the previous MATE-BP, was detected (Fig. 9) in the promyelomonocytic cell lines HL-60 and U-937, in contrast to the same complex formed in these cells and in THP-1 cells with the GRR motif (Fig. 6). Such differences could reflect multiple combinations of *trans*-acting factors that may depend on the degree of differentiation or on the particular cell lines studied.

Few mammalian cell-type-specific transcription factors have been characterized thus far. Members of a homeobox gene family (26) such as Oct-2 (42, 71) and GHF-1/Pit-1 (13) are DNA binding factors involved in the transcriptional activation of, respectively, immunoglobulin and prolactin/growth hormone genes expressed in B-lymphoid and pituitary cells. Among the factors which could achieve a muscle phenotype, three muscle-specific proteins, MyoD (18, 59), myd (51), and myogenin (73), have been identified. Another factor, hGATA-1 (62, 63), which is involved in the transcriptional activation of the globin gene, has been cloned. GATA-1 activity was found to be restricted to the erythroid cell lineage, and its cognate DNA binding site is widely distributed among promoter and enhancer sequences of genes expressed in such cells.

FcγR1 gene expression exhibits the same behavior in the sense that its activation not only depends on IFN-γ induction but is also restricted to a specific lineage. While the MATE motif is required for cell-type-specific expression, it is likely that additional elements are involved. In HeLa cells, the MATE sequence does not completely block the effect of GRR on the TK promoter in the $(GIRE)_3$-(103/78)-TK CAT construct (Fig. 7), as does the insertion of the fragment from −140 to −45. The different degrees of inhibition observed between the two constructs could depend on a particular DNA conformation involving the repetition of the AGAAAAG motif found at positions −138 to −132 and −112 to −106 and in an inverted orientation at positions −91 to −85 (Fig. 10A). The presence of the three repeated motifs within the sequence from −140 to −45 might impair the interaction of the factors with the GRR motif and those bound to the TK promoter. A change in DNA conformation by the absence of one of these motifs in the construct $(GIRE)_3$-(103/78)-TK CAT will allow only a partial inhibition of IFN-γ-induced expression. Although we cannot exclude the possibility that the sequence from −78 to −45 is playing a role in cell-type-specific expression, two cycloheximide-sensitive MATE-BP complexes are detected with probes −142/−45 and −103/−78 in THP-1 cell extracts (Fig. 8A and 9A). The different electrophoretic mobilities of these complexes could reflect another type of interaction between DNA and involved proteins. A link between GRR and MATE is indicated by the absence of the inhibitory effect of the MATE motif when cloned 3′ of the IRS motif (Fig. 7). Moreover, in cross competition experiments, an excess of the GIRE fragment reduces the binding of MATE-BP factors to the MATE motif whereas an excess of MATE in the presence of the GIRE probe does not abolish the formation of the GIRE-BP complex (data not shown). Therefore, these observations and the presence of the AGAAAAG sequence within the GRR and MATE elements suggest that GIRE-BP and MATE-BP share a factor. This factor might bind to the AGAAAAG sequence, depending on its association with proteins involved in either the IFN-γ response or cell-type-specific expression. The interaction of MATE-BP and GIRE-BP will subsequently form an active transcriptional initiation complex leading to FcγR1 gene expression.

The finding of similar sequences in promoters of several genes expressed preferentially in cells of myeloid lineage (Fig. 10B) emphasizes a role for the MATE motif in cell-type-specific expression. Recently, it has been reported (45) that the first 92 bp (containing a MATE-like motif) of the promoter of the CD11b gene was able in mobility band shift assays to form complexes specifically with myeloid-cell-specific factors. The presence of a PU box which binds an activator expressed in macrophages and B lymphocytes (32) within the MATE motif (6) suggests that this motif, if crucial for the FcγR1 expression in myeloid cells, may elicit DNA binding activities not restricted to cells of myeloid lineage.

An understanding of the molecular basis for the cell-type-restricted presence of MATE-BP and its relationship with GIRE-BP in IFN-γ-regulated gene expression should provide important insights into early events leading to the differentiation and activation of macrophages following treatment with this cytokine.

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REFERENCES

1. **Alcalay, M., F. Antolini, W. J. Van de Ven, L. Lanfrancone, F. Grignani, and P. G. Pelicci.** 1990. Characterization of human and mouse c-*fes* cDNA clones and identification of the 5′ end of the gene. Oncogene **5**:267–275.
2. **Allen, J. M., and B. Seed.** 1989. Isolation and expression of functional high affinity Fc receptor complementary DNAs. Science **243**:378–381.
3. **Barr, C. L., and G. F. Saunders.** 1991. Interferon-γ-inducible regulation of the human invariant chain gene. J. Biol. Chem. **266**:3475–3481.
4. **Basta, P. V., P. A. Sherman, and J. P.-Y. Ting.** 1988. Detailed delineation of an interferon-γ responsive elements important in human HLA-DRA gene expression in a glioblastoma multiform line. Proc. Natl. Acad. Sci. USA **85**:8618–8622.
5. **Benech, P., Y. Mory, M. Revel, and J. Chebath.** 1985. Structure of two forms of the interferon-induced (2′-5′) oligo A synthetase of human cells based on cDNAs and gene sequences. EMBO J. **4**:2249–2256.
6. **Benech, P., K. Sastry, R. R. Iyer, Q. G. Eichbaum, D. Raveh, and R. A. B. Ezekowitz.** 1992. Definition of interferon-γ-response elements in a novel human Fcγ receptor gene (FcγR1b) and characterization of the gene structure. J. Exp. Med. **176**: 1115–1123.
7. **Benech, P., M. Vigneron, D. Peretz, M. Revel, and J. Chebath.** 1987. Interferon-responsive regulatory elements in the promoter of the human 2′,5′-oligo(A) synthetase gene. Mol. Cell. Biol. **7**:4498–4504.
8. **Blanar, M. A., A. S. Baldwin, Jr., R. A. Flavell, and P. A. Sharp.** 1989. A γ-interferon induced factor that binds the interferon response element sequence of the MHC class I gene $H2K^b$. EMBO J. **8**:1139–1144.
9. **Blanar, M. A., E. C. Bottger, and R. A. Flavell.** 1988. Transcriptional activation of HLA-DRα by interferon-γ requires a trans-acting protein. Proc. Natl. Acad. Sci. USA **85**:4672–4676.
10. **Bonnerot, C., M. A. Marloie, and W. H. Fridman.** 1991.

Structure of the mouse αFcγR gene. Adv. Life Sci. **10**:53–61.
11. **Boss, J. M., and J. L. Strominger.** 1986. Regulation of a transfected human class II major histocompatibility complex gene in human fibroblast. Proc. Natl. Acad. Sci. USA **83**:9139–9143.
12. **Bottger, E. C., M. A. Blanar, and R. A. Flavell.** 1988. Cycloheximide, an inhibitor of protein synthesis, prevents g-interferon induced expression of MHC class II mRNA in a macrophage cell line. Immunogenetics **28**:215–219.
13. **Castrillo, J., M. Bodner, and M. Karin.** 1989. Purification of growth hormone specific transcription factor GHF1 containing homeobox. Science **243**:814–817.
14. **Chen, C., and H. Okayama.** 1987. High-efficiency transformation of mammalian cells by plasmid DNA. Mol. Cell. Biol. **7**:2745–2752.
15. **Cohen, B., D. Peretz, D. Vaiman, P. Benech, and J. Chebath.** 1988. Enhancer-like interferon responsive sequences of the human and murine (2′-5′) oligo A synthetase gene promoters. EMBO J. **7**:1411–1419.
16. **Collart, M. A., D. Belin, J.-D. Vassalli, S. De Kossado, and P. Vassalli.** 1986. γ-Interferon enhances macrophage transcription of the TNF/cachectin, interleukin I and urokinase genes which are controlled by short lined repressors. J. Exp. Med. **164**:2113–2118.
17. **Dale, T. C., A. M. A. Imam, I. M. Kerr, and G. Stark.** 1989. Rapid activation by interferon-α of a latent DNA binding protein present in cytoplasm of untreated cells. Proc. Natl. Acad. Sci. USA **86**:1203–1207.
18. **Davis, R. L., H. Waintraub, and A. B. Lassar.** 1987. Expression of a single transfected cDNA converts fibroblasts to myoblasts. Cell **51**:987–1000.
19. **Decker, T., D. J. Lew, Y.-S. Cheng, D. E. Levy, and J. E. Darnell, Jr.** 1989. Interaction of α- and γ-interferon in the transcriptional regulation of the gene encoding a guanylate binding protein. EMBO J. **8**:2009–2014.
20. **Decker, T., D. J. Lew, J. Mirkovitch, and J. E. Darnell, Jr.** 1991. Cytoplasmic activation of GAF, an interferon-γ regulated DNA binding factor. EMBO J. **10**:927–932.
21. **Dorn, A., B. Durand, L. Marfing, M. Le Meur, C. Benoist, and D. Mathis.** 1987. Conserved major histocompatibility complex class II boxes X and Y are transcriptional control elements and specifically bind nuclear proteins. Proc. Natl. Acad. Sci. USA **84**:6249–6253.
22. **Driggers, P. H., D. L. Ennist, S. L. Gleason, W.-H. Mak, M. S. Marks, B.-Z. Levi, J. R. Flanagan, E. Appella, and K. Ozato.** 1990. An interferon-γ regulated protein that binds the interferon inducible enhancer element of major histocompatibility complex class I gene. Proc. Natl. Acad. Sci. USA **87**:3743–3747.
23. **Farber, J. M.** 1992. A collection of mRNA species that are inducible in the RAW 264.7 mouse macrophage cell line by gamma interferon and other agents. Mol. Cell. Biol. **12**:1535–1545.
24. **Fu, X. Y., D. S. Kessler, S. A. Veals, D. E. Levy, and J. E. Darnell, Jr.** 1990. ISGF3, the transcriptional activator induced by interferon-α, consists of multiple interacting polypeptide chains. Proc. Natl. Acad. Sci. USA **87**:8555–8559.
25. **Fujita, T., H. Shubiya, T. Ohashi, K. Yamanishi, and T. Taniguchi.** 1986. Regulation of human interleukin 2 gene: functional DNA sequences in the 5′ flanking region for the gene expression in activated T lymphocytes. Cell **46**:401–407.
26. **Gehring, W. J.** 1987. Homeo boxes in the study of development. Science **236**:1245–1252.
27. **Hohn, P. A., N. C. Popescu, R. D. Hanson, G. Salvesen, and T. J. Ley.** 1989. Genomic organization and chromosomal localisation of the human cathepsin G gene. J. Biol. Chem. **264**:13412–13419.
28. **Israel, A., A. Kimura, A. Fournier, M. Fellous, and P. Kourilsky.** 1986. Interferon response element sequences potentiate activity of an enhancer in the promoter region of H-2 gene. Nature (London) **322**:743–746.
29. **Iyer, R. R.** 1991. A match set of CAT vectors for rapid mutational analysis of eukaryotic promoters and enhancers. Gene **105**:97–100.
30. **John, J., R. McKendry, S. Pellegrini, D. Flavell, I. M. Kerr, and G. R. Stark.** 1991. Isolation and characterization of a new mutant human cell line unresponsive to alpha and beta interferons. Mol. Cell. Biol. **11**:4189–4195.
31. **Kessler, D. S., S. A. Veals, X. Y. Fu, and D. E. Levy.** 1990. Interferon-α regulates nuclear translocation and DNA binding affinity of ISGF3, a multimeric transcriptional activator. Genes Dev. **4**:1753–1765.
32. **Klemsz, M. J., S. R. McKercher, A. Celada, C. Van Beveren, and R. A. Maki.** 1990. The macrophage and B cell-specific transcription factor PU-1 is related to the *ets* oncogene. Cell **61**:113–124.
33. **Kouskoff, C., R. M. Mantovani, S. M. Candelas, A. Dorn, A. Staub, B. Lisowska-Grospierre, C. Griscelli, C. O. Benoist, and D. Mathis.** 1991. NFX, a transcription factor implicated in MHC class II gene regulation. J. Immunol. **146**:3197–3204.
34. **Levy, D. E., D. S. Kessler, R. Pine, and J. E. Darnell, Jr.** 1989. Cytoplasmic activation of ISGF3, the positive regulator of interferon-α stimulated transcription reconstituted in vitro. Genes Dev. **3**:1362–1371.
35. **Levy, D. E., D. S. Kessler, R. Pine, N. Reich, and J. E. Darnell, Jr.** 1988. Interferon induced nuclear factors that bind a shared promoter element correlate with positive and negative transcriptional control. Genes Dev. **2**:383–393.
36. **Levy, D. E., A. Larner, A. Chaudhuri, L. E. Babiss, and J. E. Darnell, Jr.** 1986. Interferon stimulated transcription: isolation of an inducible gene and identification of its regulatory region. Proc. Natl. Acad. Sci. USA **83**:8929–8933.
37. **Levy, D. E., D. J. Lew, T. Decker, D. S. Kessler, and J. E. Darnell, Jr.** 1990. Synergistic interaction between interferon-α and interferon-γ through induced synthesis of one subunit of the transcription factor ISGF3. EMBO J. **9**:1105–1111.
38. **Lew, D. J., T. Decker, I. Strehlow, and J. E. Darnell.** 1991. Overlapping elements in the guanylate-binding protein gene promoter mediate transcriptional induction by alpha and gamma interferons. Mol. Cell. Biol. **11**:182–191.
39. **Luster, A. D., and J. V. Ravetch.** 1987. Genomic characterization of a gamma-interferon-inducible gene (*IP-10*) and identification of an interferon-inducible hypersensitive site. Mol. Cell. Biol. **7**:3723–3731.
39a. **Mao, C., and G. R. Stark.** Personal communication.
40. **McKendry, R., J. John, D. Flavell, M. Müller, I. M. Kerr, and G. R. Stark.** 1991. High frequency mutagenesis of human cells and characterization of a mutant unresponsive to both α and γ interferons. Proc. Natl. Acad. Sci. USA **88**:11455–11459.
41. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
41a. **Mueller, M.** Personal communication.
42. **Muller, M. M., S. Ruppert, W. Shaffner, and P. Matthias.** 1988. A cloned octamer transcription factor stimulates transcription from lymphoid specific promoter in non B cells. Nature (London) **336**:544–551.
43. **Nezu, N., K. Ryu, Y. Koide, and T. O. Yoshida.** 1990. Regulation of HLA class II molecule expression by interferon-g: the signal transduction mechanism in glioblastoma cell lines. J. Immunol. **145**:3126–3135.
44. **Pace, J. L., S. W. Russell, P. A. LeBlanc, and M. Murasko.** 1985. Cooperative effects of various classes of mouse interferons on macrophage activation for tumor cell killing. J. Immunol. **134**: 977–981.
45. **Pahl, H. L., A. G. Rosmarin, and D. G. Tenen.** 1992. Characterization of the myeloid specific CD11b promoter. Blood **79**: 865–870.
46. **Paulnock-King, D., K. C. Sizer, Y. R. Freund, P. P. Jones, and J. R. Parnes.** 1985. Coordinate induction of Ia α, β and Ii mRNA in a macrophage cell line. J. Immunol. **135**:632–636.
47. **Pearse, R. N., R. Feinman, and J. V. Ravetch.** 1991. Characterization of the promoter of the human gene encoding the high affinity IgG receptor: transcriptional induction by γ-interferon is mediated through common DNA response elements. Proc. Natl. Acad. Sci. USA **88**:11305–11309.
48. **Pellegrini, S., J. John, M. Shearer, I. M. Kerr, and G. R. Stark.**

1989. Use of a selectable marker regulated by alpha interferon to obtain mutations in the signaling pathway. Mol. Cell. Biol. **9**:4605–4612.
49. **Pestka, S., J. A. Langer, K. C. Zoon, and C. E. Samuel.** 1987. Interferons and their actions. Annu. Rev. Biochem. **56**:727–777.
50. **Pettersson, M., and W. Schaffner.** 1987. A purine rich DNA sequence motif present in SV40 and lymphotropic papovavirus binds a lymphoid specific factor and contributes to enhancer activity in lymphoid cells. Genes Dev. **1**:962–972.
51. **Pinney, D. F., S. H. Pearson-White, S. F. Konieczny, K. E. Latham, and C. P. J. Emerson.** 1988. Myogenic lineage determination and differentiation: evidence for a regulatory gene pathway. Cell **53**:781–793.
52. **Porter, A. C. G., Y. Chernajovski, T. C. Dale, C. S. Gilbert, G. R. Stark, and I. M. Kerr.** 1988. Interferon response element for the human gene 6-16. EMBO J. **7**:85–92.
53. **Reid, L. E., A. H. Brasnett, C. S. Gilbert, A. C. G. Porter, D. R. Gewert, G. R. Stark, and I. M. Kerr.** 1989. A single DNA response element can confer inducibility by both α and γ interferons. Proc. Natl. Acad. Sci. USA **86**:840–844.
54. **Revel, M., and J. Chebath.** 1986. Interferon activated genes. Trends Biochem. Sci. **11**:166–170.
55. **Schindler, C., K. Shuai, V. R. Prezioso, and J. E. Darnell.** 1992. Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. Science **257**:809–813.
56. **Skalnik, D. G., E. C. Strauss, and S. H. Orkin.** 1991. CCAAT displacement protein as a repressor of the myelomonocytic specific gp91-phox gene promoter. J. Biol. Chem. **266**:16736–16744.
57. **Sugita, K., J.-I. Miyazaki, E. Appella, and K. Ozato.** 1987. Interferons increase transcription of a major histocompatibility class I gene via a 5′ interferon consensus sequence. Mol. Cell. Biol. **7**:2625–2630.
58. **Takahashi, H., T. Nukiwa, K. Yoshimura, C. D. Quick, D. J. States, M. D. Holmes, J. Whang-Peng, T. Knutsen, and R. G. Crystal.** 1988. Structure of the human neutrophil elastase gene. J. Biol. Chem. **263**:14739–14747.
59. **Tapscott, S. J., R. L. Davis, M. J. Thayer, P. F. Cheng, H. Weintraub, and A. B. Lasar.** 1988. MyoD: a nuclear phosphoprotein requiring a Myc homology region to convert fibroblasts to myoblasts. Science **242**:405–411.
60. **Thanos, D., G. Mavrothalassitis, and J. Papamatheakis.** 1988. Multiple regulatory regions on the 5′ side of the mouse Eα gene. Proc. Natl. Acad. Sci. USA **85**:3075–3079.
61. **Tiwari, R. K., J. Kusari, R. Kumar, and G. C. Sen.** 1988. Gene induction by interferons and double-stranded RNA: selective inhibition by 2-aminopurine. Mol. Cell Biol. **8**:4289–4294.
62. **Trainor, C. D., T. Evans, G. Felsenfeld, and M. S. Boguski.** 1990. Structure and evolution of a human erythroid transcription factor. Nature (London) **343**:82–96.
63. **Tsai, S.-F., D. I. K. Martin, L. I. Zon, A. D. D'Andrea, G. G. Wong, and S. H. Orkin.** 1989. Cloning of cDNA for the major DNA binding protein of the erythroid lineage through expression in mammalian cells. Nature (London) **339**:446–451.
64. **Tsang, S. Y., M. Nakanishi, and B. M. Peterlin.** 1988. B cell specific factor and interferon-γ inducible regulation of the HLA-DRα gene. Proc. Natl. Acad. Sci. USA **85**:8598–8602.
65. **Vaiman, D., S. Pietrokovsky, B. Cohen, P. Benech, and J. Chebath.** 1990. Synergism of type I and type II interferons in stimulating the activity of the same DNA enhancer. FEBS Lett. **265**:12–16.
66. **Van de Winkel, J. G., L. K. Ernst, C. L. Anderson, and I. Chiu.** 1991. Gene organisation of the human high affinity receptor for IgG, FcγR1. J. Biol. Chem. **266**:13449–13455.
67. **Velazquez, J., M. Fellous, G. R. Stark, and S. Pellegrini.** 1992. A protein tyrosine kinase in the interferon α/β signaling pathway. Cell **70**:313–322.
68. **Weil, J., C. J. Epstein, L. B. Epstein, J. J. Sedmark, J. L. Sabran, and S. E. Grossberg.** 1983. A unique set of polypeptides is induced by γ-interferon in addition to those induced in common with α and β interferons. Nature (London) **301**:437–439.
69. **Weinshank, R. L., A. D. Luster, and J. Y. Ravetch.** 1988. Function and regulation of a murine macrophage-specific IgG Fc receptor, FcγR1. J. Exp. Med. **167**:1909–1921.
70. **Wietzerbin, J., C. Gaudelet, L. Catinot, J. Chebath, and R. Falcoff.** 1990. Synergistic effect of interferon-γ and TNF-α on antiviral activity and (2′-5′) oligo A synthetase induction in a myelomonocytic cell line. J. Leukocyte Biol. **48**:149–155.
71. **Wirth, T., L. Staudt, and D. Baltimore.** 1987. An octamer oligonucleotide upstream of the TATA motif is sufficient for lymphoid-specific promoter activity. Nature (London) **329**:174–178.
72. **Wright, T. M., and J. M. Farber.** 1991. 5′ Regulatory region of a novel cytokine gene mediates selective activation by interferon-γ. J. Exp. Med. **173**:417–422.
73. **Wright, W. E., D. A. Sassoon, and V. K. Lin.** 1989. Myogenin, a factor regulating myogenesis, has a domain homologous to MyoD. Cell **56**:607–617.
74. **Yang, Z., M. Sugawara, P. D. Ponath, L. Wessendorf, J. Banerji, Y. Li, and J. L. Strominger.** 1990. Interferon-γ response region in the promoter of the human DPA gene. Proc. Natl. Acad. Sci. USA **87**:9226–9230.
75. **Zimarino, V., and C. Wu.** 1987. Induction of sequence-specific binding of Drosophila heat shock activation protein without protein synthesis. Nature (London) **327**:727–730.