Restriction of Interferon γ Responsiveness and Basal Expression of the Myeloid Human FcγR1b Gene Is Mediated by a Functional PU.1 Site and a Transcription Initiator Consensus

By Quentin G. Eichbaum, Ravi Iyer, David P. Raveh, Carol Mathieu, and R. Alan B. Ezekowitz

From the Division of Infectious Diseases and Hematology/Oncology, Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115

Summary

The restricted expression of the human FcγR1b gene to myeloid cells is likely to be regulated by a combination of transcription factors that may not be solely expressed in myeloid cells, but act together to restrict the expression of the gene to myeloid cells. Low basal expression of the human FcγR1b gene is specifically upregulated by interferon γ (IFN-γ). A 181-bp region of 5′ flanking sequence contains several key regulatory motifs that include the extended gamma response region (XGRR) and the PIE region. The XGRR contains the 39-bp γ response region originally defined in the highly homologous FcγR1a gene. The XGRR is in close proximity to the 21-bp PIE motif that is conserved in the promoters of some other myeloid genes. The PIE motif contains a consensus site for the macrophage and B cell transcription factor, PU.1, and is adjacent to the cluster of transcription start sites. An active transcription initiator, Inr, consensus spans the start sites and appears to direct transcription initiation of this TATA-less gene. In this study, we demonstrate that the PIE region contains a functional PU.1 site that binds a human PU.1-like protein and that associated factors present in myeloid extracts also bind in this PIE region. Mutational analysis reveals an absolute requirement for an intact PU.1 box for both basal and IFN-γ inducible expression of this gene. In addition, mutations in the Inr greatly reduce basal and inducible transcription. Insertion of a strong TATA box downstream from the Inr or at −30 bp from the transcription start sites restores basal and inducible activity in the presence of a mutated PU.1 site. We also demonstrate that indeed, when the XGRR is positioned in the context of a heterologous TATA containing promoter, it is able to respond equivalently to either IFN-α or IFN-γ. However, IFN-α responsiveness does not occur in the context of the physiological FcγR1b TATA-less basal promoter. Our results suggest that a human PU.1-like factor acts as a "bridging factor" between the upstream IFN-γ enhancer and the Inr dependent preinitiation complex. These findings indicate that the structure of the basal promoter in combination with restricted activators like PU.1 are important in regulating the expression of this gene.

Much progress has been made in understanding how cell surface receptors transduce signals to the cytoplasm and nucleus of cells. Recent work on the FcγR1 gene yields insights into the mechanism of action of IFN-γ. Low basal expression of this myeloid gene is rapidly upregulated in response to IFN-γ treatment (1–3). The biological action of this cytokine is mediated via a specific 90-kD receptor (4). Ligation of the receptor with ligand results in the phosphorylation of a 91-kD cytoplasmic protein now termed Stat 91 and an 84-kD protein (5–8), a product of alternative splicing of the 91-kD gene. Both proteins are translocated to the nucleus and form part of the IFN-inducible transcription complex ISGF-3 (9–11). The Stat 91 protein recognizes an IFN-γ–activated site (GAS) which in part accounts for IFN-γ–inducible expression of some genes. It now appears that Stat 91 may be a common component of the signal transduction pathway mediated by a number of cytokines and growth factors that include PDGF, EGF, CSF-1, and IL-10 (12–15). These results raise interesting questions as to the specificity of discrete cellular responses induced by these various cytokines and growth factors that induce a common nuclear transduction pathway. The apparent explanation is that growth factors/cytokines induce both shared and distinct factors that form multimeric complexes (16).

Work from others highlighted the importance in a GAS site that binds Stat 91 (10, 17) in a 39-bp enhancer (γ re-

Q. G. Eichbaum and R. Iyer contributed equally to this work.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/94/06/1985/12 $2.00
Volume 179 June 1994 1985–1996

sponse region, GRR[1]) in the 5′ flanking region of the FcγR1a gene as necessary and sufficient for IFN-γ, but not IFN-α responsiveness (1, 18). The GRR contains motifs that resemble γ interferon response element (γIRE), X, and H box consensus that resemble elements found in the 5′ flanking region of MHC class II genes (1). Analysis of the MHC class II promoters failed to segregate minimal sequences that were sufficient for IFN-γ and IFN-α responsiveness (19). Instead the X, Y, and H box, which overlaps a GAS site, appears to be required to mediate IFN-α– and IFN-γ–induced expression of the human guanylate binding protein gene (7, 17, 18). The 3′ boundary of the GRR at positions −137 to −132 has a motif RGAAAAG that resembles a general IFN response element GAAACG (20). Further inspection of the region revealed another RGAAAAG motif at position −174 to −169 immediately upstream of the originally defined GRR. Inclusion of this 5′ RGAAAAG in the 60 bp extended GRR (XGRR) allowed an increase of IFN-γ–mediated reporter gene expression above that of the GRR (2). These findings suggested that this motif may be important in determining IFN-γ responsiveness of this gene.

A more complete analysis of FcγR1b gene expression should include an attempt to define the precise *cis* elements and *trans*-acting factors that mediate myeloid restriction. The FcγR1b IFN-γ enhancer does not contain myeloid-restricted elements, but a conserved 21-bp consensus sequence was localized between positions −84 and −104 (numbering from the ATG) and may in part account for the myeloid restricted expression of this gene (2). A similar concept has been proposed for another myeloid gene (reference 21). This *cis* motif was designated the PIE region as it contains a PU box (P) (5′-GAGGAA-3′) and an IFN responsive element (IE) (5′-RGAAAAG-3′) (2). A loose consensus of the PIE motif is represented in the 5′ flanking region of other myeloid-specific genes, but whether the motif directly contributes to lineage restriction in these genes is not clear. The motif has been identified in elastase (22), gp91-*phox* (23), *c-fes* (24), CD11b (25), *mig* (26), and cathepsin G (27). The factors binding the PIE have not yet been identified. It is known, however, that more than one factor binds the DNA in this region of the core promoter situated adjacent to the transcription start sites, and that these factors may be a combination of myeloid specific and common factors, rather than a single unique factor.

The PIE region contains a potential binding site for PU.1, a transcription factor first identified in mice, and expressed in B lymphocytes and macrophages (28). PU.1 is a member of the *Ets* family of transcription factors which includes a number of factors important in hematopoiesis, such as *Ets1*, *Erg*, *Fli-1*, and *Spi-1* (reviewed in Macleod et al. [29]). Several *Ets* family members are expressed in a tissue-specific manner, and may thus regulate tissue-specific gene expression (30). PU-1 may cooperate with other *trans*-acting proteins to enhance transcription. For example, in the murine immunoglobulin κ gene 3′ enhancer, PU.1 recruits the binding of a second B cell specific factor, NF-EM5, to a site immediately adjacent to the PU binding site (31), and the interaction between these two factors is regulated by phosphorylation (32). A complex similar to PU.1/NF-EM5 appears to be essential to the activity of the immunoglobulin λ2-4 enhancer (33). In the CD11b promoter, PU.1 has been shown to function in conjunction with the ubiquitously expressed factor Sp1 in enhancing gene expression (34). In the macrophage inflammatory protein 1a (MIP-1a) promoter, binding sites for PU.1 and C/EBP overlap, suggesting that competition for binding to this site by different factors may be functionally important in the regulation of the gene (35). PU.1 and/or Spi-B contribute to, but are not sufficient for, cell-type–specific transcriptional activation of the MIP-1a promoter.

A novel function for PU.1 may be in regulating basal transcription by binding within the core promoter. This possibility is suggested by recent work in which the activation domain of PU.1 was shown in solid phase assays to bind the general transcription factors TFIID and TFIIB that form part of the preinitiation RNA polymerase II transcription complex (36). In the FcγR1b TATA-less promoter, the presence of the PU box overlapping the initiator consensus sequence suggests that in this gene PU.1 may interact with the general transcription factors of the core promoter. In addition, PU.1 may act in the FcγR1b promoter as a "bridging factor" between the core promoter and the upstream IFN-inducible enhancer elements.

The FcγR1b promoter lacks a classical TATA and CCAAT box, but contains a motif 5-TTTTCTAATTT-3′ which conforms to a loose initiator (Inr) consensus sequence (reviewed in 37). The Inr overlaps the transcription start site and appears to have a role analogous or complementary to the TATA box in directing transcription initiation. The proteins that bind to the Inr and their interactions have not been fully elucidated. Some of the molecules that have been suggested as binding the Inr are RNA polymerase II, a transcription-associated factor, TFII-I, YY1, and E2F (37–41). In the FcγR1b promoter, the PU.1 binding site overlaps the initiator element but the interaction between the proteins binding these sites is unknown.

In this study, we show that IFN-γ responsiveness of this gene is mediated in part by discrete *cis* elements, but in addition the TATA-less structure of the basal promoter that includes the tissue restricted activator PU.1-like protein plays an important role in determining both myeloid expression and the selective IFN-γ responsiveness of this gene.

Materials and Methods

Cell Culture. The following cell lines were obtained from the American Type Culture Collection (ATCC; Rockville, MD) and cultured at 37°C in humidified air with 5% CO_2, in either RPMI 1640 medium or in DME supplemented with 10% FCS (Hyclone Laboratories, Inc., Logan, UT), 50 U of penicillin/ml and 50 μg of streptomycin/ml: U937, HL60, THP1, PLB (human promyelomonocytic cell lines), HeLa (human epithelial carcinoma cell line),

[1] *Abbreviations used in this paper:* AdML, Adenovirus major late; EMSA, electrophoretic mobility shift assay; γIRE, γ interferon response element; hGH, human growth hormone; Inr, initiator element; tk, thymidine kinase; wt, wild-type; XGRR, extended γ response region.

Daudi and Raji (human B cell lines), Jurkat (human T cell leukemic line), mouse erythroid leukemic (MEL) cell line. The mouse mast cell line (MC8), a gift from Dr. Mike Gurish (Harvard Medical School) was cultured in RPMI under similar conditions as those described above.

Preparation of Nuclear Extracts. Nuclear extracts were prepared by the procedure of Dignam et al. (42) or by the procedure of Lichtsteiner et al. (43). Since both methods of extraction produced similar results, we present data based on the Dignam method only. In vitro transcribed and translated PU.1 cDNA (a gift from Dr. R. Maki, La Jolla Cancer Research Foundation, La Jolla, CA) was prepared using the TNT Coupled Reticulocyte Lysate System supplied by Promega Corp. (Madison, WI).

Electrophoretic Mobility Shift Assays. Electrophoretic mobility shift assays were performed as previously described with some modifications (31). Oligonucleotides purified by electrophoresis were annealed (50 μg of each), endlabeled with γ-[^{32}P]dATP, and the unincorporated label separated by passage through a DNA grade Sephadex G-50 column (Pharmacia LKB Biotechnology, Piscataway, NJ). 5 μg of nuclear extract was incubated on ice for 30 min with 10,000 cpm ($\sim$0.251 g) of probe in a 30-μl reaction mixture containing 12 mM Hepes (pH 7.9), 0.5 mM EDTA, 5 mM $MgCl_2$, 10% (vol/vol) glycerol, 1 mM dithiothreitol (DTT), 60 mM KCl, 0.05% NP40, and 21 μg of poly (dI-dC) (Pharmacia LKB Biotechnology). Mixtures were preincubated on ice for 5–10 min before addition of the probe. In competition experiments, unlabeled annealed oligonucleotides (100–200-fold excess) were added together with the nonspecific competitor poly (dI-dC) for 15 min before the addition of the probe, followed by a further 15-min incubation on ice. After incubation, the samples were fractionated at 4°C on 5% polyacrylamide gels (acrylamide/bisacrylamide, 40:1) in 11 mM Tris-borate (pH 8.0) and 1 mM EDTA, for 1 h at 35 mA.

Antibody supershift assays were performed by preincubating nuclear extracts or in vitro translated PU.1 protein for 15 min on ice with anti-PU.1 antibody or preimmune serum before addition of the probe for an additional 15 min, followed by electrophoresis. The anti-PU.1 antiserum (1297), raised against the NH_2-terminal domain of the protein, was obtained from Dr. R. Maki.

Transient Transfection Assays. Plasmid constructs were prepared by annealing complementary, phosphorylated, synthetic oligonucleotides containing either the wild-type (wt) XGRR sequence or its 5′ or 3′ deletion. The annealed double stranded fragments were constructed to give overhangs compatible with multiple cloning sites present in the HSV-thymidine kinase (tk) promoter containing CAT reporter gene vector, pBRAMS cat 2 (44) into which these were cloned. The point mutation in the construct XGRRΔ165-152 was constructed by annealing synthetic oligonucleotides of the sequence: 5′-GATCCAGGTATGAGCATGGGAAAAGCATCCATGGTCCATGGGAGATGTATTTCCCAGAAAAGGAACATG-3′; 5′-GATCCATGTTCCTTTTCTGGGAAATACATCTCCCATGGACCATGGATGCTTTTCCCATGCTCATACCTG-3′. Hela cells (10-cm dishes) were grown to 70% confluence ($\sim 10^7$ cells) in complete medium (DME with 10% FCS) and transiently transfected by the DEAE-dextran method. Each dish received 2 ml of transfection mixture (DME + 10% NuSerum [Hyclone Laboratories Inc., Logan, UT] with 250 μg/ml of DEAE-Dextran + 30 μg of relevant test plasmid + 5 μg of the constitutively expressing human growth hormone plasmid, CMV-GH [Nichols Institute, San Juan Capistrano, CA] as the internal control for transfection efficiency). After a brief "glycerol shock" step, the cells were incubated in complete medium for 24 h. The individual aliquots of each originally transfected dish was then subjected to either no further treatment or a 4-h stimulation with 300 U/ml of IFN-γ (Actimmune; Genentech Inc., South San Francisco, CA) or 600 U/ml of IFN-α (Roferon 2a; Hoffmann-La Roche, Nutley, NJ). The cells were then harvested and a cytoplasmic extract was prepared. The hGH activity (ηg/ml) in the culture supernate was assayed using the Allegro hGH assay kit supplied by Nichols Institute according to manufacturer's instructions. The CAT enzyme activity was assayed according to the protocol suggested by Promega Corp. with the exception that the volume of cell extracts used was adjusted to equivalent hGH units so as to normalize for minor variations in transfection efficiency between dishes. The reactions were extracted in ethyl acetate, spotted onto silica gel–coated TLC plates, and developed in chloroform/methanol (98:2 vol/vol). The plates were then exposed and analyzed on a phosphor imager with densitometric quantitation of the acetylated chloramphenicol spots using image analysis software.

In other experiments, promoter region constructs (−189 to −7) were transiently transfected into cells by electroporation. 10^7 cells were washed twice with PBS and once in IMDM, and then resuspended at a concentration of 10^7 cells/0.5 ml IMDM for electroporation (Gene Pulser; Bio-Rad Laboratories, Richmond, CA). The conditions for electroportion of the different cell lines were as follows: U937 (300 V, 9,601 F), HeLa (150 V, 9601 F), and Raji cells (200 V, 9601 F). As a control for the efficiency of transfection, 2 μg of a plasmid containing a human GH gene sequence linked to the CMV promoter was cotransfected into the cells. The luciferase activity of the experimental plasmids was normalized to 1 μg of hGH/ml of medium as measured by radioimmunoassay (Nichol's Institute). Immediately after electroporation, the cells were incubated on ice for 15 min and then returned to the culture medium for 8 h before being harvested. IFN-stimulated cells received 300 U of human IFN-γ (Actimmune; a kind gift from Dr. S. Kramer, Genentech, Inc.) after the first 4 h of incubation. Luciferase assays were performed as described. Luciferase activity was expressed as millivolts per microgram of growth hormone produced per ml in the transfection medium (mV/μg GH/ml).

Luciferase Assay. Luciferase activity was measured in cells harvested 8 h after transfection. Cells were pelleted by centrifugation at 500 *g* for 5 min and 100 μl of the supernatant was retained for measurement of the cotransfected hGH reporter gene activity. The pellet was washed twice in PBS and resuspended in 500 μl of lysis buffer (25 mM glycylglycine, 15 mM $MgSO_4$, 4 mM EGTA, 1 mM DTT, 1% Triton X-100; pH 7.8). Samples were vortexed for 2 min, centrifuged at 1,200 *g* at 4°C for 5 min, and the supernatant snap frozen at −80°C. For luminometry, samples were thawed to room temperature and 300 μl of sample buffer (25 mM glycylglycine, 15 mM $MgSO_4$, 4 mM EGTA, 1 mM DTT; pH 7.8) was added to 100 μl of cell sample, followed by addition of fresh 1 mM ATP and 100 μl of luciferin. Luciferase activity was read in millivolts on a BioOrbit Luminometer 1251 (LKB, Wallac, Finland).

Plasmid Constructs. The luciferase reporter gene vectors pGLO1 and pGLO2 were derived, respectively, from the vectors pBRAMs-cat1 and pBRAMS-cat2 (containing the HSV-tk promoter) (44), by replacing the CAT gene of the latter vectors with the luciferase gene sequences obtained from the vector pGEM-LUC (Promega Corp.).

The 3-bp linker scan mutations of the PIE region were prepared by annealing sets of oligonucleotides containing three base pair transversion mutations at serial positions over the region −104 to −79. BamHI and NotI cloning sites were included at the ends of the annealed oligonucleotides for subcloning of the fragments into pGLO2.

The 10-bp mutation linker scan constructs were made by oligonucleotide directed PCR mutagenesis by substituting a 10-bp linker

containing a BglII site (sequence 5'-AGATCTCAGA-3') for the wt sequence between bp −114 and −44 of the FcγR1 promoter (45). The PCR fragments were cloned into the luciferase vector pGLO1 and each construct was sequenced to confirm correct positioning of the oligonucleotide linker.

The transfection control plasmid CMV-LUC construct was made by replacing the growth hormone gene sequence of the plasmid CMV-GH, with the luciferase gene obtained from the vector pGEMluc (Promega Corp.).

The plasmid CMV-GH was a construct of a human growth hormone sequence cloned into the plasmid pUC12 driven by the CMV promoter (a gift from Dr. Len Zon, Harvard Medical School).

Results

Mutational 10-BP Linker Scan of the Basal Promoter Identifies Critical Sequences in the PIE and INR Region. To more precisely define functional motifs that are required for FcγR1b basal promoter activity, we carried out a mutational scan using a 10-bp linker (-5'-AGATCTCAGA-3') over the region −104 to −44 that was encompassed by the DNase I footprints of the region (shown schematically in Fig. 1). Seven linker constructs (Fig. 2) inserted downstream of the 60-bp IFN-γ–responsive region (XGRR) in the context of a luciferase reporter gene were transfected into U937 cells and assayed for luciferase activity with and without IFN-γ treatment. A series of three consecutive mutations between −104 and −74 resulted in a large decrease in luciferase activity that was not increased when cells were treated with IFN-γ. Almost complete loss of activity was observed in the construct −94/−84 that incorporated the consensus PU.1 binding site on the lower strand (5'-GAGGAA-3'). The mutation −104/−94 that incorporated the upstream region of the PIE and 4 bp at the 5' of the PU motif resulted in a reduction of activity to about 25% of wt activity. A mutation that spanned

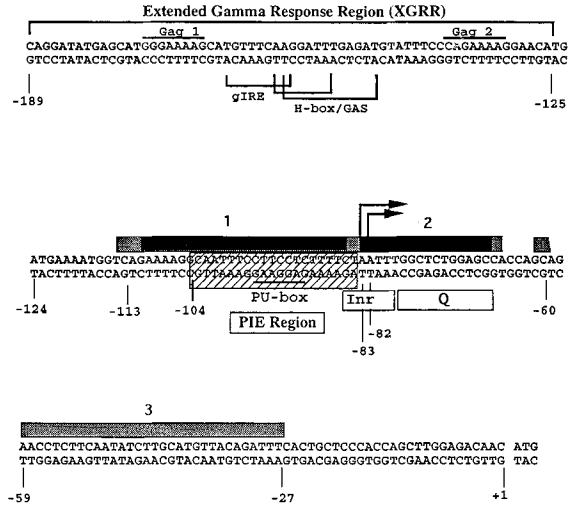


Figure 1. Schematic representation of the FcγR1 promoter indicating the location of the (XGRR), the PIE region, Inr, Q region and the transcription start sites (*arrows*). The three regions protected (1, 2, and 3) in DNase 1 footprinting assays (data not shown) are indicated by bars above the sequence. The solid bar indicates strongly footprinted regions and the hatched bars represent regions of weaker protection.

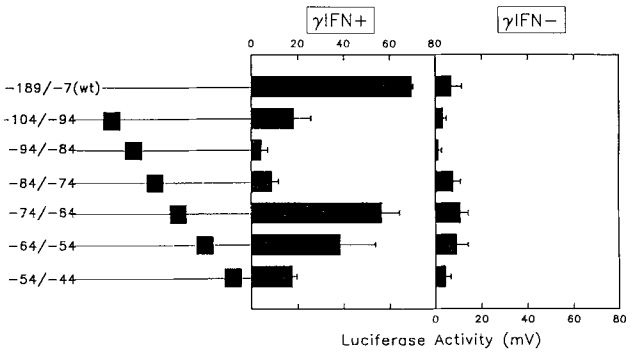


Figure 2. Identification of critical *cis* elements contained in the FcγR1b promoter. (*A*) Transient transfections of 10-bp linker scan mutation constructs in the luciferase reporter gene vector pGLO1 and U937 cells that were either stimulated for 4 h with human recombinant IFN-γ or untreated. In the diagram, the straight lines represent the wt sequences whereas the mutated linker sequence is represented by the filled in square. The position of the mutant linker in the promoter is indicated on the left of the diagram. Three independent experiments were performed and the average luciferase activities and standard errors were calculated. Luciferase activity, reported in mV, was normalized to 1 μg of growth hormone produced per ml of medium by a cotransfected CMV-GH plasmid (Materials and Methods). Control plasmids used in each transfection assay are described in Materials and Methods.

the transcription initiator −84/−74, resulted in 10% of luciferase activity after IFN-γ treatment of transfected cells. In addition, a region −54/−44 downstream of the transcription start sites also appeared to contribute to the expression of the reporter gene. Our next goal was to define whether these functionally important regions were sites for DNA binding proteins, in particular, a PU.1-like protein and possible associated factors. To investigate this possibility, we performed electrophoretic mobility shift assays (EMSA).

EMSAs Show That PU.1 Binds in the PIE Region. A radiolabeled oligonucleotide probe that represented the PIE region containing the PU box was incubated with nuclear extracts isolated from 10 different cell lines of hematopoietic and epithelial origin and retarded complexes were compared in a standard gel shift assay. Extracts from myeloid cell lines U937, THP1, HL60, and PLB consistently contained the binding activities designated B1 and B2 as shown in Fig. 3. Complexes B1 and B2 were both consistently present in only the myeloid nuclear extracts, whereas only B1 was present in MEL and Raji extracts and B2 was present in Mast (MC8) and Daudi extracts. HeLa and Jurkat extracts did not retard any specific complexes. A slower mobility complex, running above B1, was seen in all complexes and appeared to be nonspecific as it could not be competed out with any of the cold competitor oligonucleotides (not shown). The B3 complex was present only in myeloid extracts, but was relatively unstable and most likely represents a proteolytic fragment of B1.

Several lines of evidence indicate that a factor with identical or similar binding properties to that of PU.1 may account for complex B1. The presence of the complex in only myeloid and B cell extracts is consistent with the known pattern of expression of PU.1. In addition, we show that in vitro translated PU.1 protein forms a complex in gel shift

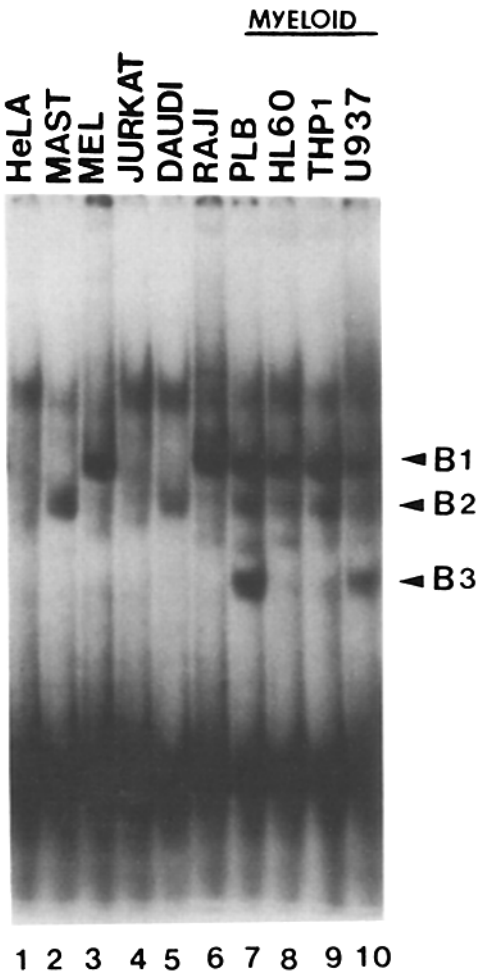


Figure 3. EMSA showing formation of DNA-protein complexes between the PIE region, oligonucleotide probe, and nuclear extracts of various myeloid and non myeloid cell lines. The probe was prepared as described in Materials and Methods and the binding reactions with 5 μg of nuclear extract were carried out on ice for 30 min in the presence of 2 μg of poly (dI-dC). The complexes were separated by electrophoresis through a 5% polyacrylamide gel (35 mA for 2 h) at 4°C in 1× Tris-buffered EDTA buffer. Arrows on the right indicate the positions of the three complexes B1, B2, and B3.

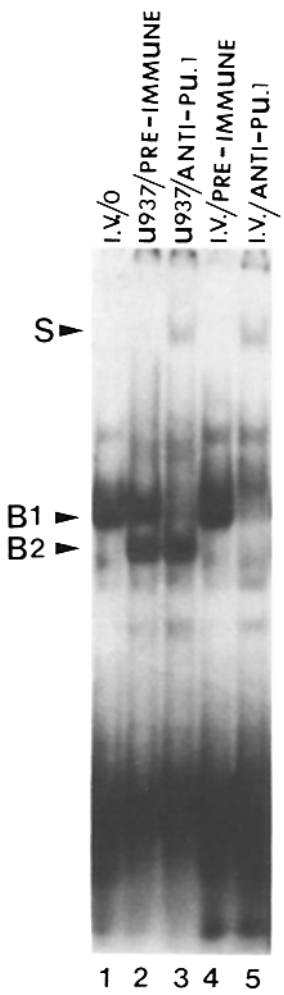
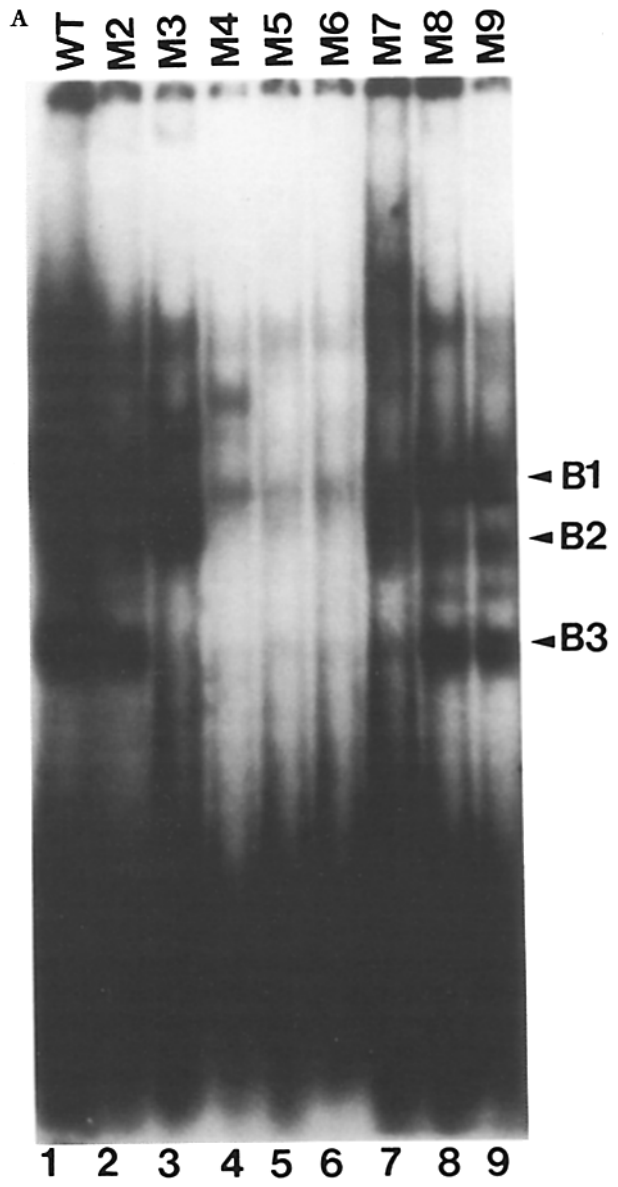


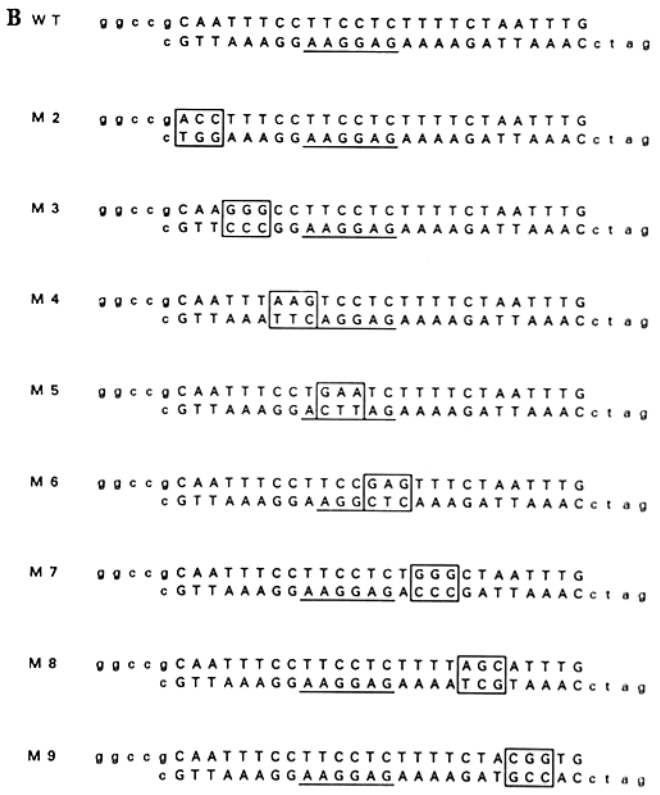
Figure 4. Anti-PU.1 antibody supershift analysis of U937 nuclear extracts and in vitro translated PU.1. In vitro translated PU.1 or nuclear extracts of U937 cells were preincubated on ice for 15 min with either preimmune serum or anti-PU.1 serum before a further 15-min incubation with the PIE oligonucleotide probe and EMSA under standard conditions. (Lane *1*) In vitro translated PU.1 without antibody; (lane *2*) U937 extracts with preimmune serum; (lane *3*) U937 extracts with anti-PU.1 antibody; (lane *4*) in vitro translated PU.1 with preimmune serum; (lane *5*) in vitro translated PU.1 with anti-PU.1 serum.

assays with the PIE oligonucleotide that comigrates with complex B1 present in U937 nuclear extracts (Fig. 4, lane *1*). Preincubation of U937 nuclear extract or in vitro translated PU.1 protein with anti-PU.1 antiserum that recognizes the NH_2 terminus of the protein resulted in a supershifted mobility of the B1 complex and in vitro translated PU.1 to similar positions in the gel (Fig. 4, lanes *3* and *5*). Preincubation with preimmune serum had no effect on the mobility of the complexes (Fig. 4; lanes *2* and *4*). These results suggest that a human protein that is closely related to the murine PU.1 binds to the PU box within the PIE region of the FcγR1b core promoter.

3-BP Linker Scan of PIE Region Indicates That PU.1 Is Necessary for Basal Promoter Activity. As the methylation interference patterns of the two complexes were equivocal (results not shown), we performed a linker scan of consecutive 3-bp mutations across the PIE region and the transcription start sites between basepairs −104 and −79 in order to gain some understanding of the critical base pairs contacted within this region. The unmutated wt and eight additional mutated annealed oligonucleotides containing NotI and BamHI sites at alternate ends for cloning into the luciferase reporter gene vectors (pGLO1 and 2) were assayed in parallel for both in vitro binding and transcriptional activity (Fig. 5, *A* and *C*). The plasmid pGLO2 containing the tk promoter was used since constructs in the promoterless vector pGLO1 showed little activity (results not shown). The low activity may in part be due to the exclusion in these constructs of the region Q immediately downstream of the transcription start sites.

The results of gel shift assays of the 3-bp PIE mutants (Fig. 5 *A*) and the transfections of these constructs into U937 cells (Fig. 5 *C*) indicated a correlation between mutations within PU box (M4, M5, M6) that resulted in a loss of binding of complexes B1 and B2 and of the unstable complex B3 with a decrease of between 70 and 50% in reporter gene expression. Mutation M3 resulted in an altered pattern of binding (Fig. 5 *A*, lane *3*) that correlated with a 50% reduction in reporter gene activity in transfections (Fig. 5 *C*). The M2 mutation that defines the 5′ boundary of the PIE region also encompassed a potential binding site for the *myb* transcrip-





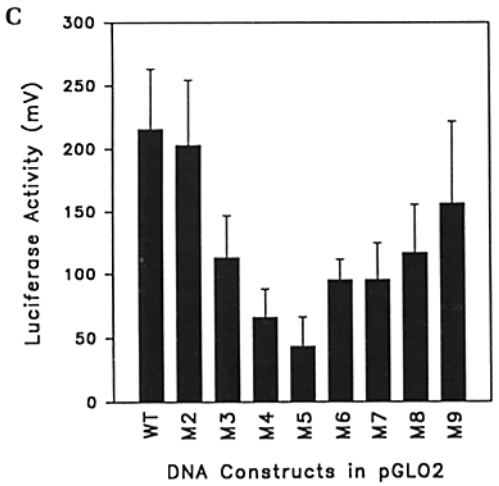


Figure 5. 3 bp mutational scan of the PIE region to identify DNA binding motifs. Sets of complementary oligonucleotides incorporating 3-bp transversion mutations were annealed and used as probes in gel retardation and transient transfection assays to determine factor binding motifs in the PIE region. (*A*) Gel retardation assay showing binding of nuclear extracts of U937 cells to wt and mutated oligonucleotide probes (M2-M9) of the PIE region. The probes used in each reaction are indicated above the lanes, and the complexes B1, B2, and B3 are shown on the right. (*B*) Schematic representation of the oligonucleotides representing the 3-bp mutational scan across the PIE region. The mutated base pairs are indicated in boxes. Lower case letters depict the BamHI and NotI restriction sites used in cloning the mutated sequences into pGLO2 for transfection into U937 and HeLa cells. (*C*) Transcriptional activity of the 3-bp mutants in U937. The mutated oligonucleotide probes of the PIE region were cloned into the luciferase reporter gene vector pGLO2 containing the heterologous thymidine kinase promoter. The results represent the mean of three independent experiments.

tion factor of macrophages (GGCAAT) or of the transcription factor C/EBP (G/CCAAT) and consistently showed a decreased intensity in binding of complex B2 (Fig. 5 *A*, lane *2*), but was not accompanied by a decrease in basal reporter gene expression. Mutation M7 that altered the contiguous three adenines of the classical PU consensus sequence 5'-AAA-GAGGAA-5' (Fig. 5 *B*) resulted in a marked reduction in binding intensity of complexes B1, B2, and B3 in gel shift assays (Fig. 5 *A*, lane 7) and a 50% reduction in reporter gene activity in transfections (Fig. 5 *C*). Mutant M8 disrupted the 3' end of the PIE retained gel shift binding activity, but displayed a 55% decrease in reporter gene expression (Fig. 5 *C*). These results provided further evidence for the importance of an intact PU.1 site in the basal expression of the gene, but also suggested that this region is a composite binding site.

The PU.1 Site and the Initiator (Inr) Are Required for Basal and IFN-γ–Inducible Activity. Low basal expression of the FcγR1b gene is rapidly and specifically upregulated by IFN-γ treatment. RNA polymerase II directed transcription of this gene occurs in the absence of a TATA box. Although the promoter lacks a TATA box, it contains a motif spanning the transcription start sites that is compatible with that of an initiator element (Inr) (5'-TTTTTCTAATTT-3') located at the transcription start sites (37). The 10-bp linker mutation scan indicated that region −104 to −74 contained the PU.1 box and the Inr consensus resulted in a reduction in IFN-γ–inducible reporter gene expression. To more precisely define the relative roles of these motifs, we introduced point mutations in the PU.1 box (189/7δPU) and the Inr (189/7 δInr), respectively (Fig. 1). Mutation of either site resulted in a marked reduction in both basal and IFN-γ inducible expression and there appeared to be an absolute requirement for an intact PU.1 site. Interestingly, mutations in the GCAAT sequence at the 5' end of the PIE and immediately downstream from the Inr (189/7δQ) resulted in a marked reduction in transcriptional activity (Fig. 6, *A* and *B*, construct 189/7ΔCAAT). These results suggested that both the PIE region, which includes the PU site and the Inr and Q sites, are integral components of the basal promoter.

Responsiveness to IFN-γ and IFN-α Occurs in the Context of a Heterologous TATA Box. The XGRR was subcloned into a plasmid that contained the chloramphenicol acetyl transferase reporter gene CAT and the HSV-tk basal promoter (44). Transient transfection of this plasmid into the human epithelial HeLa cell line followed by a 4-h treatment of the cells with IFN-γ resulted in a 25-fold induction of CAT activity (Fig. 7), as previously described (2). However, IFN-α treatment of the cells transfected with this construct also resulted in a 25-fold induction of CAT activity. To determine whether distinct or overlapping *cis* elements accounted for the IFN-α– and IFN-γ–mediated inducible expression, a series of 5' and 3' deletion constructs were assembled in the tk-CAT vector, pBRAMScat2 (Fig. 7). The construct, 189/147 tk-CAT, lacks the 3' RGAAAAG motif, but includes the upstream RGAAAAG element and the central γIRE, X, H box/GAS site (Fig. 7). This plasmid retained the IFN-α inducible CAT activity, but was unresponsive to induction with

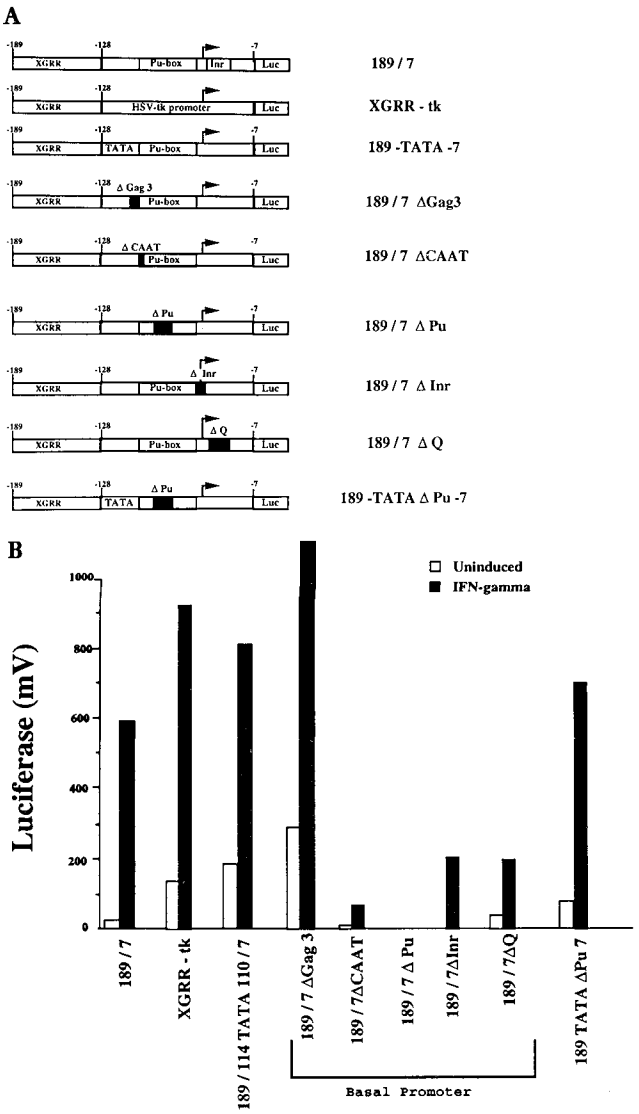


Figure 6. Mutations of the PIE region (including the PU box), Inr consensus sequence and the Q site reduce basal promoter activity and IFN-γ inducibility. (*A*) Schematic representations of mutations made in the promoter. (*B*) Constructs depicted above were transfected into U937 cells and assayed for luciferase activity, both with and without induction of the cell by IFN-γ. In addition, the effect of creating a canonical adenovirus major late promoter TATA box sequence by a 3-bp mutation change upstream of an unmutated PU box (189/114 TATA 110/7) was tested for the ability to restore transcriptional activity and IFN inducibility. 189/7 is wt sequence from −189 to −7; XGRR-tk is the extended IFN-γ response element cloned into pGLO2 under control of the thymidine kinase promoter; 189/7dGag3 is a construct containing mutations in the third -GAAAG- motif of the promoter that lies immediately 5' of the PIE region; 189/7dCAAT is a mutation in the CAAT motif situated at the 5' end of the PIE region; 189/7dPu is a construct in which all 6 bp of the PU box are mutated; 189/7dInr is the mutated sequence; 189/7dQ is the mutated Q motif; 189 TATA dPU is a sequence containing an inserted AdML promoter TATA box and a mutated PU box. The results are representative of three independent experiments.

IFN-γ (Fig. 7, lane 189/147). Further deletion of the H box/GAS sequence with retention of an intact γIRE and the 5' RGAAAAG motif in the construct 189/158 tk-CAT

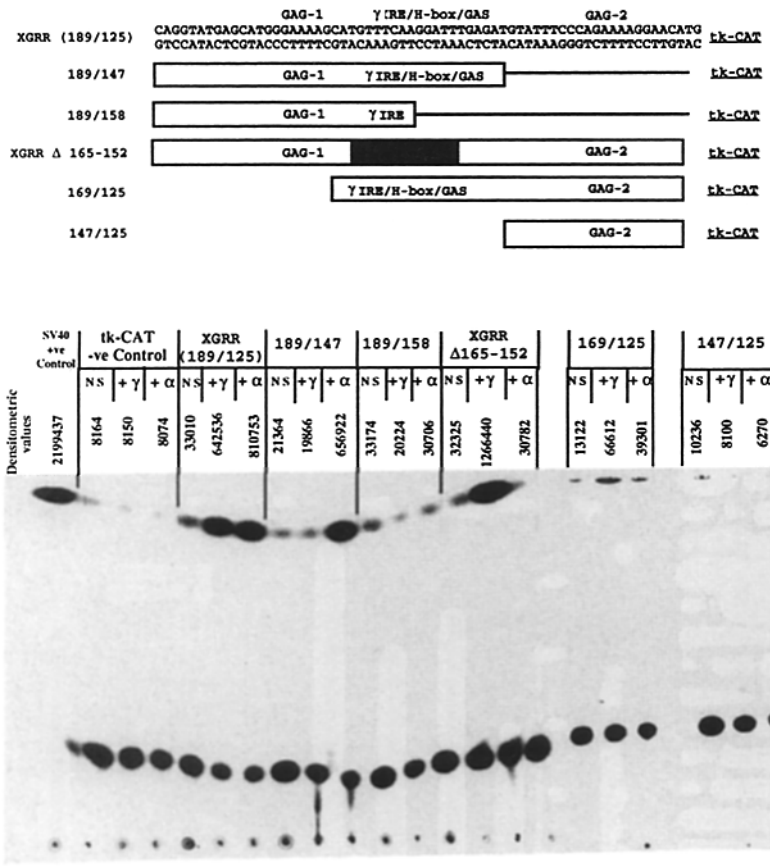


Figure 7. CAT assay of transiently transfected HeLa cells with various deletion/mutant constructs of the XGRR in the context of the TATA-containing heterologous HSV-tk promoter-CAT reporter gene plasmid, pBRAMScat2. NS, No Stimulation; +γ, 4 h. IFN-γ stimulation (300 U/ml); +α, 4 hours. IFN-α stimulation (600 U/ml). The densitometric values in absolute numbers are indicated in each lane.

resulted in a loss of IFN-α responsiveness (Fig. 7, lane 189/158). Transfection of Hela cells with a construct lacking the upstream 5′ RGAAAAG motif, but containing the γIRE/H box/GAS site, and the downstream RGAAAAG site, (GRR) resulted in modest IFN-α responsiveness but also retained the ability, albeit reduced, to respond to IFN-γ when compared to wild type XGRR (Fig. 2, lane 169/125). By contrast, the IFN-γ effect appears to be dependent upon the two RGAAAAG motifs as transfection of a reporter plasmid that contains a disrupted γIRE, X, H Box/GAS site flanked by these two elements is able to induce 40-fold induction of CAT activity after IFN-γ treatment (Fig. 7; lane XGRR Δ165-152).

The observation that the XGRR was unable to mediate an IFN-α–inducible effect when placed upstream of the endogenous gene promoter, and yet displayed dual cytokine responsiveness when in the context of the heterologous TATA containing HSV-tk promoter suggested that the structure of the basal promoter may play an important role in restricting cytokine responsiveness. To investigate the role of the basal promoter, we cloned the fragment −189 to −7, containing the XGRR and the endogenous FcγR1b TATA-less promoter into a luciferase reporter plasmid (Fig. 6 *A*). Transient transfection of this construct into U937 cells did not result in any significant IFN-α inducible reporter gene expression whereas a 25-fold induction was observed after IFN-γ treatment (Fig. 8). By contrast, transfection of XGRR-tk-luc (containing the XGRR upstream of the heterologous tk promoter-luciferase reporter plasmid; Fig. 6 *A*) resulted in significant IFN-γ and IFN-α inducible expression (Fig. 8). This suggested (*a*) that U937 cells were capable of responding to both IFN-γ and IFN-α; and (*b*) that the ability of the XGRR to mediate dual cytokine responsiveness was dependent on the nature of the downstream promoter. One alternative explanation was that the endogenous TATA-less promoter in the construct 189/7-luc contained a repressor element that silenced the IFN-α inducibility of the upstream XGRR. To test this hypothesis, we transfected U937 cells with the construct 189/7-tk-luc (Fig. 6 *A*) in which the HSV-tk promoter was placed downstream of the XGRR and the endogenous FcγR1b basal promoter. It was reasoned that in the event of active repression, the lack of IFN-α inducibility would not be relieved by providing a TATA-based promoter downstream. Alternatively, if the IFN-α responsiveness was dependent on a TATA box, addition of the HSV-tk promoter downstream should result in significant IFN-α inducibility. The results (Fig. 8; lane 189/7-tk-luc) show equivalent responsiveness to IFN-γ and IFN-α. A potential confounding variable is that the HSV-tk promoter also contains a Y-box element. The Y box has been shown to complement the activity of the H and X boxes in the interferon inducibility of MHC class II gene expression (19). Since the XGRR also contains H and X box-like sequences, it was possible that the observed IFN-α responsiveness was due to the presence of the Y box and not the TATA element of the HSV-tk promoter. To evaluate this possibility, we introduced the

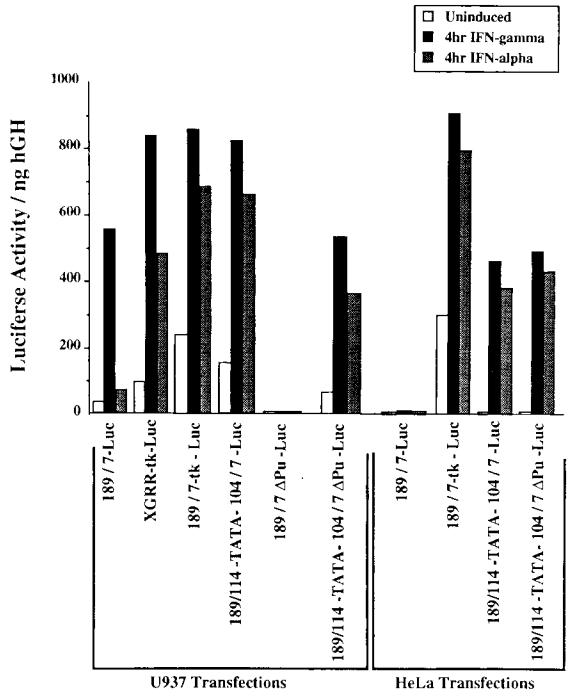


Figure 8. (*A*) Structure of the various luciferase constructs tested in *B*. (*B*) Luciferase activity assay of transiently transfected U937 and HeLa cells with the FcγR1b promoter/XGRR region constructs, assayed with/without IFN-γ or IFN-α stimulation. U937 transfections: (Lane *1*) wt promoter/enhancer (189/7), 189/7-luc; (Lane *2*) XGRR in the context of the HSV-tk promoter, XGRR-tk-luc; (Lane *3*) wt promoter/enhancer (189/7) in the context of the HSV-tk promoter, 189/7-tk-luc; (Lane *4*) wt promoter/enhancer (189/7) with AdML TATA box introduced at −113 to −105, 189/114-TATA-104/7-luc; (Lane *5*) wt promoter/enhancer (189/7) with point mutation of the PU-box (−96 to −91), 189/7 ΔPu-luc; (Lane *6*) wt promoter/enhancer with PU-box mutation with AdML TATA box introduced at −114 to −105, 189/114-TATA-104/7 ΔPu-luc. HeLa transfections; (Lane *7*) wt promoter/enhancer (189/7), 189/7-luc; (Lane *8*) wt promoter/enhancer (189/7) in the context of the HSV-tk promoter, 189/7-tk-luc; (Lane *9*) wt promoter/enhancer (189/7) with AdML TATA box introduced at −113 to −105, 189/114-TATA-104/7-luc; (Lane *10*) wt promoter/enhancer with PU-box mutation with AdML TATA box introduced at −114 to −105, 189/114-TATA-104/7 ΔPu-luc.

Adenovirus major late TATA box (AdML TATA) into the endogenous FcγR1b promoter at bp −113 (Figs. 1 and 6 *A*). This map position is −30 bp from the major transcription start site cluster at −82 and −83 bp. In addition, the site at which the TATA box was introduced did not contain an important regulatory motif since introduction of a restriction site at that position did not alter either basal or IFN-γ–inducible luciferase expression (results not shown). Transient transfection of 189/114-TATA-104/7luc into U937 cells resulted in comparable induction of luciferase activity after both IFN-γ and IFN-α treatment.

The implication that the endogenous TATA-less basal promoter was apparently restrictive for the type of the cytokine inducibility mediated by the XGRR prompted us to examine whether the insertion of a TATA box may also permit expression of FcγR1b promoter in nonmyeloid cells. In prior studies, we had observed that placing the XGRR in the context of the endogenous promoter restricted its activity in transient transfection systems to myeloid cells (2). We wished to see whether the constructs 189/7 tk-luc and 189/114-TATA-104/7-Luc (Fig. 6 *a*) could restore the ability of the XGRR to function in nonmyeloid (Hela) cells. Transient transfection of these constructs into HeLa cells followed by IFN-γ or IFN-α stimulation resulted in significant inducible responses to both cytokines (Fig. 8). This indicated that the TATA-less structure of the endogenous FcγR1b basal promoter was not only restrictive for the type of cytokine inducibility mediated by the XGRR, it also appeared to play a role in restricting myeloid specificity of reporter gene expression as assayed by transient transfection. It is pertinent to note that we have not performed primer extension assays to determine the precise transcription start sites of the reporter gene mRNA in these experiments due to the difficulties of obtaining good quality RNA from the subpopulation of transiently transfected cells that actually received the transfected plasmid.

Interestingly, the effects of the mutation in the PU.1 box that abrogate expression in the context of the physiologically relevant promoter could be reversed by introducing an AdML TATA-box upstream of the PIE region as in construct 189/114-TATA-104/7-ΔPu-Luc (Figs. 7 and 8). The presence of the TATA box in the above construct also had the effect of restoring IFN-α inducibility in addition to enabling reporter gene expression in HeLa cells as well as U937 cells.

Discussion

We present data that demonstrates the presence of a functional PU.1 binding site located within a 21-bp conserved promoter element (PIE) of the myeloid restricted FcγR1 gene. We have also identified a loose Inr spanning two transcription start sites that serves a critical function in mediating transcription of this TATA-less gene. Mutation of either the PU.1 motif or the Inr element abrogates basal transcription and both of these sites are essential for the IFN-γ inducibility of the gene. Insertion of a strong TATA box permits interferon α responsiveness in addition to enhanced IFN-γ–mediated transcription. The TATA containing plasmids are active in nonmyeloid cells even in the context of an intact PU.1 site and Inr consensus. We hypothesize that the core promoter of the FcγR1 may play an active role in regulating the gene's expression.

A PU.1 binding site (GAGGAA) was identified on the lower strand of the PIE region and was shown to be functionally active. A 10-bp linker mutation scan across the footprinted region (−104/−44) confirmed that the PU box was functionally active. A 3-bp mutation scan across the PIE region more precisely identified the core nucleotides of the PU motif. There was a direct correlation between in vitro binding activity of the mutant constructs as determined by gel shift assays and in vivo expression in transient transfections of the same reporter gene constructs. Furthermore, evidence for a human PU.1-like protein that bound to the PU box was derived from competition experiments (results not shown). Excess unlabeled oligonucleotides containing the PU consensus motif GAGGAA inhibited binding of the PIE region probe to the B1 complex. The B1 complex comigrated with in vitro translated PU.1 protein in a gel shift assay and both

of these complexes could be supershifted to a similar position on the gel by anti-PU.1 antiserum.

PU.1 has been shown to function in association with other factors (31, 33, 34, 36, 46). In the mouse immunoglobulin K 3′ enhancer for example, PU.1 recruits a second B cell restricted factor, NF-EM5 (31). It is therefore possible that the PIE region provides a composite binding platform for a PU.1-like factor and associated proteins. Some support for this idea arises from the 3-bp linker scan of the PIE region (Fig. 5, *A* and *B*). Mutations within the core GAGGAA resulted in loss of binding of complexes B1 and B2 (Fig. 5, *A*, lanes *4*, *5*, and *6*), whereas mutations of the peripheral nucleotides resulted in an altered pattern and intensity of binding of the complexes (Fig. 5 *A*, lanes *3* and 7). It is possible that if there is a distinct associated factor, its binding may be dependent on the prior binding of PU.1 as has been shown to be the case in the immunoglobulin λ2-4 enhancer (33).

The B2 complex most likely represents a degradation product of B1. The middle of the PU.1 protein contains a so-called PEST region composed of the amino acids proline (P), glutamic acid (E), serine (S), and threonine (T) which is susceptible to protease degradation (28, 31). The anti-PU.1 serum directed against the NH_2 terminus of the protein resulted in a supershift of only complex B1 in band shift assays. The failure of the antiserum to recognize the lower B2 complex may be consistent with its being a degradation product (47). An association between B1 and B2 could be tested using a polyclonal antiserum raised against the whole PU.1 protein. Failure to supershift complex B2 would indicate that it is a distinct complex. Carvalho and Derse (47) showed that different anti-PU.1 sera resulted in different patterns of supershifted complexes. Our results indicate the presence of three complexes in the myeloid cell lines tested (U937, PLB, HL60, THP1). Perez et al. (48) describe a similar set of complexes (MATE-BP1, MATE-BP2, MATE-BP3) that bind this region of the FcγR1b promoter in extracts isolated from THP1 cells. However, these authors do not detect the slower migrating complexes in extracts from U937 and HL60 cells. One explanation for this difference is proteolysis of the slower migrating complexes.

Since PU.1 is expressed in both B cells and macrophages, it seems that it cannot alone account for the myeloid specificity of the FcγR1b gene. Other factors must be involved to achieve such lineage specificity. These putative factors may lie outside the PIE region. We show the sequence GCAAT overlapping the 5′ end of the PIE is functionally active (Fig. 6 *B*, construct ΔCAAT), and it is possible that a factor binding this sequence may act in combination with PU.1 to achieve lineage restriction. A similar motif, G/CCAAT, is known to bind members of the C/EBP family of proteins in myeloid cells (21, 49).

The insertion of a strong (AdML) TATA box at a position 30 bp upstream of the major transcription start site or in the form of a tk promoter (in pGLO2) abrogates the tissue restricted expression of the promoter and allows it to function in nonmyeloid cells. Furthermore, we show that insertion of such a TATA box renders the promoter responsive not only to IFN-γ, but also to IFN-α. Both lineage-restricted expression of the gene and its selective responsiveness to IFN-γ are thus abrogated by insertion of a TATA box into the endogenous TATA-less promoter. The PU motif is essential for both the IFN responsiveness of the gene and core promoter activity.

Recently, it has been reported that the tumor suppressor protein p53 specifically represses transcription from TATA-containing promoters, but has no effect on initiator-mediated transcription (50). It is conceivable that in the FcγR1 TATA-less promoter, the PU.1 factor exerts a repressive effect on the initiator element through interaction with the basal transcription factors. Our results may therefore be explained by the proposition that a cell-mediated activator, like PU.1, when placed in the context of a relatively weak transcription initiator may act as a bridging factor between the upstream IFN-γ enhancer and the preinitiation complex. PU.1 is able to interact in vitro with the general transcription factor TFIID as has recently shown been shown (36). As Inr directed transcription in the absence of a TATA box requires TFIID consisting of TATA binding protein and its associated factors (transcription-associated factors) (40, 41, 51, 52), link between these key elements can be envisioned. In the absence of a TATA box, the ability of the Inr to direct assembly of the preinitiation complex depends on its strength (37). Strong initiators, like that of the Tdt promoter, are capable of initiation in the absence of a functional TATA box (37, 53, 54). However, weak initiators have been shown to require, and indeed function in cooperation with, a functional TATA element (reviewed in reference 53 and references therein). The FcγR1b gene promoter has a weak Inr consensus and no functional TATA box. In this case, the ability of PU.1 and PU.1-associated factor(s) to interact with and stabilize TFIID on the basal promoter may be critical in allowing assembly of the preinitiation complex upon the weak Inr. Such a scheme may also account for the myeloid specific restriction of FcγR1b gene expression. Assembly of basal transcription factors on the TATA-less promoter would be dependent on the existence of both PU.1 as well as PU.1 associated proteins. The observation that the FcγR1 gene is not expressed in B cells may be due to the unavailability of these additional PU.1 associated in B lymphocytes. The mechanisms by which a TATA box relieves the PU.1/Inr restriction of interferon responsiveness is not known, but these findings bring into focus the potentially important role of the structure of the basal promoter in determining the restriction of gene expression.

We are grateful to Dr. Richard Maki for providing invaluable reagents and to Drs. Stuart Orkin, Jeff Ravetch, Dan Tenen, Renate Schiebe, and members of our laboratory for helpful discussions. We especially appreciate Dr. Steven Smale's invaluable advice and critical review of the manuscript.

This work is supported by National Institutes of Health grant RO1 AI-23786 and MO1 RR-02171. Q. Eichbaum is a Cancer Research Institute Postdoctoral Fellow and R. A. B. Ezekowitz in an Established Investigator of the American Heart Association.

Address correspondence to Dr. R. Alan B. Ezekowitz, Division of Infectious Diseases and Hematology/Oncology, Children's Hospital, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115.

Received for publication 17 November 1993 and in revised form 14 February 1994.

References

1. Pearse, R.N., R. Feinman, and J.V. Ravetch. 1991. Characterization of the human Fc-γ R1 gene promoter: transcriptional induction by γ interferon is mediated through common DNA response elements. *Proc. Natl. Acad. Sci. USA.* 88:11305.
2. Benech, P.D., K. Sastry, R.R. Iyer, Q.G. Eichbaum, D.P. Raveh, and R.A.B. Ezekowitz. 1992. Definition of interferon γ–response elements in a novel human Fcγ receptor (FcγRIb) and characterization of the gene structure. *J. Exp. Med.* 176:1115.
3. Wilson, K.C., and D.S. Finbloom. 1992. Interferon γ rapidly induces in human monocytes a DNA-binding factor that recognizes the gamma response region within the promoter of the gene for the high-affinity Fcγ receptor. *Proc. Natl. Acad. Sci. USA.* 89:11964.
4. Auget, M., Z. Dembic, and G. Merlan. 1988. Molecular cloning and expression of the human interferon γ receptor. *Cell.* 55:273.
5. Schindler, C., K. Shuai, V.R. Prezioso, and J.E. Darnell, Jr. 1992. Interferon-γ dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. *Science (Wash. DC).* 257:809.
6. Shuai, K., C. Schindler, V.R. Prezioso, and J.E. Darnell. 1992. Activation of transcription by IFN-γ: tyrosine phosphorylation of a 91-kD DNA binding protein. *Science (Wash. DC).* 258:1808.
7. Shuai, K., G.R. Stark, I.M. Kerr, and J.E. Darnell, Jr. 1993. A single phosphotyrosine residue of Stat91 required for gene activation by interferon-γ. *Science (Wash. DC).* 261:1744.
8. Fu, X., C. Schindler, T. Improta, R. Aebersold, and J.E. Darnell. 1992. The proteins of ISGF-3, the interferon α-induced transcriptional activator, define a gene family involved in signal transduction. *Proc. Natl. Acad. Sci. USA.* 89:7840.
9. Kessler, D.S., S.A. Veals, X.-Y. Fu, and D.E. Levy. 1990. Inteferon-α regulates nuclear translocation and DNA-binding affinity of ISGF3, a multimeric transcriptional activator. *Genes & Dev.* 4:1753.
10. 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.
11. Dale, T.C., A.M.A. Imam, I.M. Kerr, and G.R. Stark. 1989. Rapid activation by interferon α of a latent DNA-binding protein present in the cytoplasm of untreated cells. *Proc. Natl. Acad. Sci. USA.* 86:1203.
12. Larner, A.C., M. David, G.M. Feldman, K. Igarashi, R.H. Hackett, D.S.A. Webb, S.M. Sweitzer, E.F. Petricoin III, and D.S. Finbloom. 1993. Tyrosine phosphorylation of DNA binding proteins by multiple cytokines. *Science (Wash. DC).* 261:1730.
13. Silvennoinen, O., C. Schindler, J. Schlessinger, and D.E. Levy. 1993. Ras-independent growth factor signaling by transcription factor tyrosine phosphorylation. *Science (Wash. DC).* 261:1736.
14. Ruff-Jamison, S., K. Chen, and S. Cohen. 1993. Induction by EGF and interferon-γ to tyrosine phosphorylated DNA binding proteins in mouse liver nuclei. *Science (Wash. DC).* 261:1733.
15. Sadowski, H.B., K. Shuai, J.E. Darnell, Jr., and M.Z. Gilman. 1993. A common nuclear signal transduction pathway activated by growth factor and cytokine receptors. *Science (Wash. DC).* 261:1739.
16. Montminy, M. 1993. Trying on a new pair of SH2s. *Science (Wash. DC).* 261:1694.
17. 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 (Eur. Mol. Biol. Organ.) J.* 10:927.
18. Pearse, R.N., R. Feinman, K. Shuai, J.E. Darnell, Jr., and J.V. Ravetch. 1993. Interferon γ-induced transcription of the high-affinity Fc receptor for IgG requires assembly of a complex that includes the 91-kDa subunit of transcription factor ISGF3. *Proc. Natl. Acad. Sci. USA.* 90:4314.
19. Benoist, C., and D. Mathis. 1990. Regulation of major histocompatibility complex class II genes: X, Y, and other letters of the alphabet. *Annu. Rev. Immunol.* 9:457.
20. LaMarco, K.L., and S.L. McKnight. 1989. Purification of a set of cellular polypeptides that bind to purine rich cis-regulatory elements of herpes simplex virus immediate early genes. *Genes & Dev.* 3:1372.
21. Suzow, J., and A.D. Friedman. 1993. The murine myeloperoxidase promoter contains several functional elements, one of which binds a cell type-restricted transcription factor, myeloid nuclear factor 1 (MyNF1). *Mol. Cell. Biol.* 13:2141.
22. 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.
23. Skalnick, 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.
24. Alcalay, M., F. Antolini, W.J. Van de Ven, L. Lanfrancone, F. Grignani, and P.G. Pelicci. 1990. Characterization of human and mouse c-fos cDNA clones and identification of the 5′ end of the gene. *Oncogene.* 5:267.
25. Galas, D., and A. Schmitz. 1978. DNase foot-printing: a simple method for the detection of protein-DNA binding specificity. *Nucleic Acids Res.* 5:3157.
26. 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.
27. Hohn, P.A., N.C. Popuescu, R.D. Hanson, G. Salvesen, and T.J. Ley. 1989. Genomic organization and chromosomal localization of the human cathepsin G gene. *J. Biol. Chem.* 264:13412.
28. Klemsz, M.J., S.R. McKercher, A. Celada, C. van Beveren, and R.A. Maki. 1990. The macrophage and B cell-specific tran-

scription factor Pu.1 is related to its oncogene. *Cell.* 61:113.
29. Macleod, K., D. Leprince, and D. Stehelin. 1992. The ets gene family. *TIBS (Trends Biochem. Sci.).* 199:251.
30. Karim, F.D., L.D. Umess, C.S. Thummel, M.J. Klemsz, S.R. McKercher, A. Celada, C. van Beveren, R.A. Maki, C.V. Gunther, J.A. Nye, and B.I. Graves. 1990. The ETS-domain: a new DNA-binding motif that recognizes a purine-rich core DNA sequence. *Genes & Dev.* 4:1451.
31. Pongubala, J.M.R., S. Nagulapalli, M.J. Klemsz, S.R. McKercher, R.A. Maki, and M.L. Atchison. 1992. PU.1 recruits a second nuclear factor to a site important for immunoglobulin $\kappa 3'$ enhancer activity. *Mol. Cell. Biol.* 12:368.
32. Pongubala, J.M.R. C. van Beveren, S. Nagulapalli, M.J. Klemsz, S.R. McKercher, R.A. Maki, and M.L. Atchison. 1993. Effect of PU.1 phosphorylation on interaction with NF-EM5 and transcriptional activation. *Science (Wash. DC).* 259: 1622.
33. Eisenbeis, C.F., H. Singh, and U. Storb. 1993. PU.1 is a component of a multiprotein complex which binds an essential site in the murine immunoglobulin δ 2-4 enhancer. *Mol. Cell. Biol.* 13:6452.
34. Chen, H.M., H.L. Pahl, R.J. Scheibe, D.E. Zhang, and D.G. Tenen. 1993. The SpI transcription factor binds the CD11b promoter specifically in myeloid cells *in vivo* and is essential for myeloid-specific promoter activity. *J. Biol. Chem.* 268:8230.
35. Grove, M., and M. Plumb. 1993. C/EBP, NF-kB, and c-Ets family members and transcriptional regulation of the cell-specific and inducible macrophage inflammatory protein 1 α immediate-early gene. *Mol. Cell. Biol.* 13:5278.
36. Hagemeier, C., A.J. Bannister, A. Cook, and T. Kouzarides. 1993. The activation domain of transcription factor PU.1 binds the retinoblastoma (RB) protein and the transcription factor TFIID *in vitro*: RB shows sequence similarity to TFIID and TFIIB. *Proc. Natl. Acad. Sci. USA.* 90:1580.
37. Smale, S.T. 1994. Core promoter architecture for eukaryotic protein-coding genes. *In* Transcription: Mechanisms and Regulation. J. Conaway and R. Conaway, editors. Raven Press, New York. 63–81.
38. Zenzie-Gregory, B., A. O'Shea-Greenfield, and S.T. Smale. 1992. Similar mechanisms for transcription initiation mediated through a TATA box or an initiator element. *J. Biol. Chem.* 267:2823.
39. Zenzie-Gregory, B., A. Khachi, I.P. Garraway, and S.T. Smale. 1993. Mechanism of initiator-mediated transcription: evidence for a functional interaction between the TATA-binding protein and DNA in the absence of a specific recognition sequence. *Mol. Cell. Biol.* 13:3841.
40. Roy, A.L., M. Meisterernst, P. Pognonec, and R.C. Roeder. 1991. Cooperative interaction of an initiator-binding transcription initiation factor and the helix-loop helix-activator USF. *Nature (Lond.).* 354:245.
41. Shi, Y., E. Seto, L.-S. Cheng, and T. Shenk. 1991. Transcriptional repression by YY1 a human GLI-Kruppel-related protein and relief of repression by Adenovirus E1a protein. *Cell.* 67:377.
42. Dignam, J.E., P.I. Martin, B.S. Shastry, and R.G. Roeder. 1983. Eukaryotic gene transcription with purified components. *Methods Enzymol.* 101:582.
43. Lichtsteiner, S., J. Wuarin, and U. Schibler. 1987. DNA-binding proteins on the promoter of the mouse albumin gene. *Cell.* 51:963.
44. Iyer, R.R. 1991. A matched set of CAT vectors for rapid mutation analysis of eukaryotic promoters and enhancers. *Gene.* 105:97.
45. Zaret, K.S., J.-K. Liu, and C.M. DiPersio. 1990. Site-directed mutagenesis reveals a liver transcription factor essential for the albumin transcriptional enhancer. *Proc. Natl. Acad. Sci. USA.* 87:5469.
46. Galson, D.L., J.O. Hensold, T.R. Bishop, M. Schalling, A.D. D'Andrea, C. Jones, P.E. Auron, and D.E. Housman. 1993. Mouse β-globin DNA-binding protein B1 is identical to a proto-oncogene, the transcription factor Spi-1/PU.1, and is restricted in expression to hematopoietic cells and the testis. *Mol. Cell. Biol.* 13:2929.
47. Carvalho, M., and D. Derse. 1993. The PU.1/Spi-1 proto-oncogene is a transcriptional regulator of a lentivirus promoter. *J. Virol.* 67:3885.
48. Perez, C., J. Wietzerbin, and P.D. Benech. 1993. Two cis-DNA elements involved in myeloid-cell-specific expression and γ interferon activation of the human high-affinity Fcγ receptor gene: a novel interferon regulatory mechanism. *Mol. Cell. Biol.* 13:2182.
49. Scott, L.M., C.I. Civin, P. Rorth, and A.D. Friedman. 1992. A novel expression pattern of three C/EBP family members in differentiating myelomonocytic cells. *Blood.* 80:1725.
50. Mack, D.H., J. Vartikar, J.M. Pipas, and L.A. Laimins. 1993. Specific repression of TATA-mediated but not initiator-mediated transcription by wild-type p53. *Nature (Lond.).* 363:281.
51. Seto, E., Y. Shi, and T. Shenk. 1991. YYI is an initiator sequence-binding protein that directs and activates transcription *in vitro*. *Nature (Lond.).* 354:241.
52. Cormack, B.P., and K. Struhl. 1992. The TATA-binding protein is required for transcription by all three nuclear RNA polymerases in yeast cells. *Cell.* 69:685.
53. Hernandez, N. 1993. TBP, a universal eukaryotic transcription factor? *Genes & Dev.* 7:1291.
54. Smale, S.T., and D. Baltimore. 1989. The 'initiator' as a transcription control element. *Cell.* 57:103.