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Identification of a Region Which Directs the Monocytic Activity of the Colony-Stimulating Factor 1 (Macrophage Colony-Stimulating Factor) Receptor Promoter and Binds PEBP2/CBF (AML1)

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The receptor for the macrophage colony-stimulating factor (or colony-stimulating factor 1 [CSF-1]) is expressed from different promoters in monocytic cells and placental trophoblasts. We have demonstrated that the monocyte-specific expression of the CSF-1 receptor is regulated at the level of transcription by a tissue-specific promoter whose activity is stimulated by the monocyte/B-cell-specific transcription factor PU.1 (D.-E. Zhang, C. J. Hetherington, H.-M. Chen, and D. G. Tenen, Mol. Cell. Biol. 14:373–381, 1994). Here we report that the tissue specificity of this promoter is also mediated by sequences in a region II (bp −88 to −59), which lies 10 bp upstream from the PU.1-binding site. When analyzed by DNase footprinting, region II was protected preferentially in monocytic cells. Electrophoretic mobility shift assays confirmed that region II interacts specifically with nuclear proteins from monocytic cells. Two gel shift complexes (Mono A and Mono B) were formed with separate sequence elements within this region. Competition and supershift experiments indicate that Mono B contains a member of the polyomavirus enhancer-binding protein 2/core-binding factor (PEBP2/CBF) family, which includes the *AML1* gene product, while Mono A is a distinct complex preferentially expressed in monocytic cells. Promoter constructs with mutations in these sequence elements were no longer expressed specifically in monocytes. Furthermore, multimerized region II sequence elements enhanced the activity of a heterologous thymidine kinase promoter in monocytic cells but not other cell types tested. These results indicate that the monocyte/B-cell-specific transcription factor PU.1 and the Mono A and Mono B protein complexes act in concert to regulate monocyte-specific transcription of the CSF-1 receptor.

During hematopoiesis, pluripotent stem cells in the bone marrow give rise to committed progenitor cells that eventually form lineage-specific mature circulating blood cells, including erythrocytes, T and B lymphocytes, megakaryocytes, granulocytes, and monocytes/macrophages (12). Characterization of the transcription factors regulating the lineage commitment of hematopoietic progenitor cells is an important step toward understanding the mechanisms of cellular differentiation and organ development. For example, the transcription factor GATA-1 has been shown to play important regulatory roles in progenitor cells differentiating in the erythrocyte lineage (17), but less is known about the molecular basis of monocytic lineage commitment.

Many experiments have demonstrated that the macrophage colony-stimulating factor (colony-stimulating factor 1 [CSF-1]) is required for the differentiation, proliferation, and survival of monocytic phagocytes (20, 22). The CSF-1 receptor, which is identical to the product of the c-*fms* proto-oncogene, is a 150-kDa transmembrane glycoprotein with an intracellular tyrosine kinase domain (20). The expression of the CSF-1 receptor is normally restricted to two tissues, monocytes and placental trophoblasts, in which it is regulated by different promoters (18, 24). In monocytes, transcription starts at multiple sites within 200 bp of the ATG start codon. In placental trophoblastic cells, transcription starts 26 kb upstream and includes an additional noncoding exon.

CSF-1 receptor mRNA and surface expression of the protein can be detected in the early stages of monocytic differentiation and increases dramatically with increasing cell maturity within this lineage (19, 25). Because the expression of the CSF-1 receptor is developmentally regulated and restricted to the monocytic cells, it can be used as a model with which to study the mechanisms regulating commitment to the monocytic lineage. We have previously demonstrated by nuclear run-on analysis that tissue-specific expression of the CSF-1 receptor is regulated at a transcriptional level in monocytic cells (30). In addition, sequences 500 bp upstream of the transcriptional origins in monocytic cells contain tissue-specific promoter activity (18, 30). The B-cell- and myeloid cell-specific transcription factor PU.1 (Spi1) binds to sequences within this promoter region and plays an important role in this tissue-specific promoter activity (30).

Although important for expression of the CSF-1 receptor gene by monocytes, PU.1 is also found in B lymphocytes (6). Mutation of the PU.1-binding site decreases CSF-1 receptor promoter activity in a tissue-specific fashion, although the mutated promoter still retains residual tissue-specific activity (30). This finding suggests that there are probably other monocyte-specific elements that direct tissue specific expression of the CSF-1 receptor gene in combination with PU.1. In

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the present study, we investigated this possibility by promoter deletion analysis and DNA binding experiments. We report the discovery of two other transcription complexes that bind just upstream of the PU.1 site and demonstrate the importance of their binding sites for CSF-1 receptor monocyte-specific promoter activity. One of the complexes is related to the PEBP2/CBF family member AML1 (9), which is located at the breakpoint of a common chromosome translocation in acute myelogenous leukemia (AML) and has recently been reported to play a role in the myeloid cell-specific expression of the myeloperoxidase gene (11). The other complex is expressed preferentially but not exclusively in monocytic cells. These two complexes bind to a region which comprises a monocyte-specific enhancer element which, along with the adjacent PU.1-binding site, direct monocyte-specific expression of the CSF-1 receptor gene.

MATERIALS AND METHODS

Cell culture. Human monoblastic U937 (ATCC CRL 1593; American Type Culture Collection, Rockville, Md.), B-lymphoblastic BJA-B, and T-lymphoblastic Jurkat (ATCC TIB 152) and REX cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and 2 mM L-glutamine. Human promonocytic THP-1 cells (ATCC TIB 202) were grown in RPMI 1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, and 2×10^{-5} M 2-mercaptoethanol. The human Mono Mac 6 cells were propagated as described previously (32). Human cervical carcinoma HeLa cells (ATCC CCL 2) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% newborn calf serum and 2 mM L-glutamine.

Transient transfections. For analysis of deletion constructs, U937 and Jurkat cells were transfected by electroporation in serum-free Iscove's modified Eagle's medium at 960 μF and 170 V. The detailed transfection procedures were as described previously (14) except that the final cell concentration was 10^8 cells/ml, and 0.2 ml of cells was aliquoted into each cuvette. These cells were maintained in Iscove's medium containing 15% fetal calf serum before harvest. Cells were harvested 8 h post-transfection in 1 ml of lysis buffer, and luciferase assays were performed as described previously (1, 14). The transfection efficiency was normalized on the basis of the level of the secreted form of the human placental alkaline phosphatase (SEAP) activity (13), and the data were calculated in relative light units per unit of SEAP activity. For analysis of other constructs, transfection experiments were performed as described previously (30).

Deletion constructs of the CSF-1 receptor proximal promoter. Plasmid a pB5/−480 was fashioned by cloning the proximal (monocytic) human CSF-1 receptor promoter 550-bp *Sac*I fragment into the promoterless and enhancerless luciferase vector pGL2-basic (Promega). Assigning the most 5′ major transcription start site in monocytic cells as +1 (18), this *Sac*I fragment contains CSF-1 receptor sequences from −480 to +70 bp (18). Plasmids pB5/−294, pB5/−214, pB5/−85, and pB5/−25 were constructed by using PCR with the pB5/−480 template. The primers used for PCR were 5′-GATCTGCC CGGGCCACCTGATCA-3′ (−294), 5′-TCTCTTCTCCCGG GACCCCTTGA-3′ (−214), 5′-CTTAAAGACCCGGGATTT CCAAA-3′ (−85), and 5′-AGGATCAGCCCGGGGAGGAG GAA-3′ (−25) for the 5′ ends and 5′-CTTTATGTTTTTGG CGTCTTCCA-3′ for the 3′ end. These promoter fragments were placed in plasmid pGL2-basic digested with restriction endonucleases *Sac*I and *Xmn*I. The additional plasmids pB5/−407 and pB5/−185 were constructed by truncation of the 5′-proximal promoter fragment of pB5/−480, using *Nsi*I-*Kpn*I or *Apa*I-*Kpn*I double restriction digests.

Nuclear extracts. Cells were grown to the log phase and harvested for nuclear protein extraction. Nuclear extracts for DNase I footprinting were prepared as previously described (31). Nuclear extracts for band shift were prepared by the method of Dignam et al. (3) with all of the proteinase inhibitors as described for DNase I footprinting (31).

DNase I footprinting. A 540-bp fragment extending from bp −416 to +124 of the CSF-1 receptor promoter was digested with *Bam*HI and *Xho*I to release the promoter fragment from the vector pXP2 (30). ^{32}P labeling at *Xho*I site of the DNA fragment and performance of the DNase I footprinting were as described previously (31).

EMSA. ^{32}P-labeled double-stranded oligonucleotides for electrophoretic mobility shift assay (EMSA) were prepared as previously described (30). All double-stranded oligonucleotides have *Sal*I and *Xho*I sticky ends at the upstream and downstream ends, respectively. DNA binding and electrophoresis conditions were as described previously (30). CSF-1 receptor promoter oligonucleotide (0.25 ng) was end labeled with [γ-^{32}P]ATP and incubated with 1 μg of double-stranded poly(dI-dC) and 50 ng of unlabeled coding (single)-stranded bp −88 to −59 oligonucleotide in the presence of nuclear protein. (The reactions presented in Fig. 3 do not contain unlabeled single-stranded oligonucleotide.) For competition experiments, an excess of double-stranded unlabeled oligonucleotides (exact amounts are specified in the figure legends) were added to the binding reaction mixture together with the other components. For supershift experiments, 1 μl of an antiserum raised against a 17-amino-acid N-terminal peptide from AML1 (7) was added to the reaction mixture 10 min prior to addition of the probe. As a control, 1 μl of an antiserum raised against transcription factor Oct-1 (Santa Cruz Biotechnology, Inc., Santa Cruz, Calif.) was used instead.

Site-directed mutagenesis of region II. The wild-type CSF-1 receptor promoter-luciferase constructs p540M-CSF-R-luc and pM-CSF-R-luc were described previously (30). Mutated CSF-1 receptor promoter fragments were generated by PCR, with plasmid p540M-CSF-R-luc as a template. Primer A (5′-CGGGATCCAGATATGCATTACTTTGGAGATTCCA AGG-3′) was used with either primer C (5′-GGGGTACCTG GGTCTTTAAGAAG-3′) or primer E (5′-TCGAGCAGGTA CCTTACAGAGTTTGGAAATCTTG-3′) to generate PCR fragment 1 or 3, respectively. Primer B (5′-GGGGTACCCCT CGGTGGGGAAGTGGCA-3′) was used with either primer D (5′-TCGACAGGTACCATAAACTCTGTGGTTGCCTT GC-3′), primer F (5′-GGGGTACCTGCCTAGCTAAAAG G-3′), or primer G (5′-GGGGTACCGATCAGCCCAAG-3′) to generate PCR fragment 2, 4, or 5, respectively. PCR fragments 1 and 3 were digested with *Bam*HI and *Kpn*I. PCR fragments 2, 4, and 5 were digested with *Kpn*I and *Sac*I. Digested PCR fragments 1 and 2 or 3 and 4 were ligated with *Bam*HI- and *Sac*I-digested vector pXP2 to construct pM-CSF-R(MA)-luc or pM-CSF-R(MB)-luc, respectively. Digested PCR fragments 1 and 5 were ligated with *Bam*HI- and *Sac*I-digested vector pXP2 to construct pM-CSF-R(DD)-luc. The sequences of the mutant constructs were confirmed by the dideoxy-chain termination method. The mutated constructs pM-CSF-R(MA)-luc and pM-CSF-R(MB)-luc changed the sequence at bp −86 to −79 from AGATTTCC to GGTAC-CAT and the sequence at bp −71 to −62 from GTGGTT-GCCT to CTAAGGTACC. The construct pM-CSF-R(DD)-luc deleted bp −86 to −37 from the wild-type CSF-1 receptor promoter.

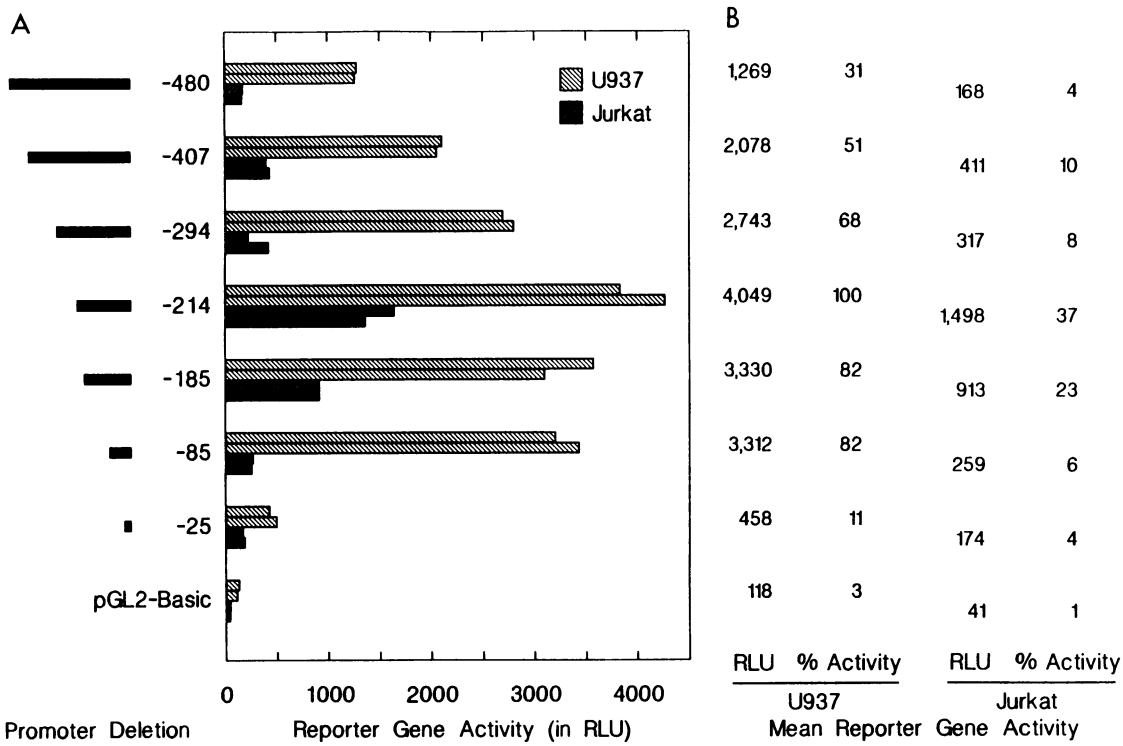


FIG. 1. Transient transfection analysis of CSF-1 receptor promoter activity. (A) Schematic representations of the CSF-1 receptor 5′ upstream sequence-luciferase constructs. These constructs and the promoterless pGL2-basic vector alone were transfected into U937 monocytic leukemia cells and Jurkat T-lymphoid leukemia cells in two independent experiments. The reporter gene activity of each construct in relative light units (RLU) is shown as a bar chart. To control for differences in transfection efficiency, we cotransfected a plasmid containing a SEAP gene driven by the Rous sarcoma virus long terminal repeat. (B) Mean reporter gene activity in RLU per unit of SEAP activity tabulated along with the percent activity relative to the activity of the −214 promoter in U937 cells.

Heterologous promoter analysis. The head-to-tail-ligated tetramer and hexamer of the region II wild-type double-stranded oligonucleotide (−88 to −59) were inserted into the *Sal*I site of pBluescript(KS−), digested with *Xho*I and *Hin*dIII, blunt ended, and religated into *Sma*I-digested pT81-luc to form p(Mono)$_4$T81-luc, p(Mono)$_6$T81-luc, and p(Mono)$_{6R}$ T81-luc. p(Mono)$_4$T81-luc and p(Mono)$_6$T81-luc contain the tetramer and hexamer of region II in the 5′ site of the thymidine kinase promoter in pT81-luc in the same orientation as in the parental promoter. p(Mono)$_{6R}$T81-luc contains the hexamer of region II in the reversed orientation. These constructs were transfected into monocytic and nonmonocytic cell lines to compare the promoter activity relative to that of the basal herpes simplex virus thymidine kinase promoter construct pT81-luc (10, 30).

RESULTS

Deletion analysis of the CSF-1 receptor promoter demonstrates that as little as 85 bp of 5′ flanking DNA directs monocytic expression. Previous studies of the human CSF-1 receptor promoter indicated that the proximal promoter adjacent to exon 2 displayed specificity for cells of the myeloid/macrophage lineage (18, 30). To further examine the activity of the proximal promoter and to identify the *cis*-acting regulatory elements responsible for tissue specificity, we generated a series of deletion mutants and tested them by a transient transfection assay in U937 and Jurkat cells, using luciferase reporter constructs. The construct containing 480 bp upstream of the 5′ major transcription initiation site in human monocytic cells (18) was expressed at approximately 10-fold-higher levels in U937 (monocytic) cells than in Jurkat (T-lymphocytic) cells (Fig. 1). The activity of the promoter in both cell lines increased with deletions extending to −214 bp, suggesting the loss of sequences mediating transcriptional repression. A construct retaining only 85 bp of the promoter upstream of the initiation site retained most of the promoter activity of the −214 bp construct, as well as the tissue-restricted pattern of expression, with 15-fold-higher levels of activity in U937 cells than in Jurkat cells. These results suggest that there are both negative and positive *cis*-acting elements in the 480-bp region immediately upstream of the major CSF-1 receptor initiation site that are responsible for the CSF-1 receptor promoter activity and indicate the presence of important positive elements in a fragment containing only 85 bp of 5′ flanking DNA relative to the major start site of transcription.

Nuclear proteins from monocytic cells interact with the CSF-1 receptor promoter in a cell-type-specific manner. *cis*-acting elements direct the promoter's activity through interaction with ubiquitous and tissue specific *trans*-acting factors. To further analyze the factors mediating monocyte-specific CSF-1 receptor promoter activity, we performed DNase I footprinting with a DNA fragment containing the CSF-1 receptor promoter and nuclear proteins from Mono Mac 6 and U937 monocytic leukemia cells and HeLa cervical carcinoma epithelial cells. As shown in Fig. 2, three regions of the CSF-1 receptor promoter (bp −150 to +60) interacted with nuclear proteins from monocytic cells. When coding-strand-labeled DNA fragments were used as probes, nuclear proteins from both monocytic and nonmonocytic cells protected region I, including the

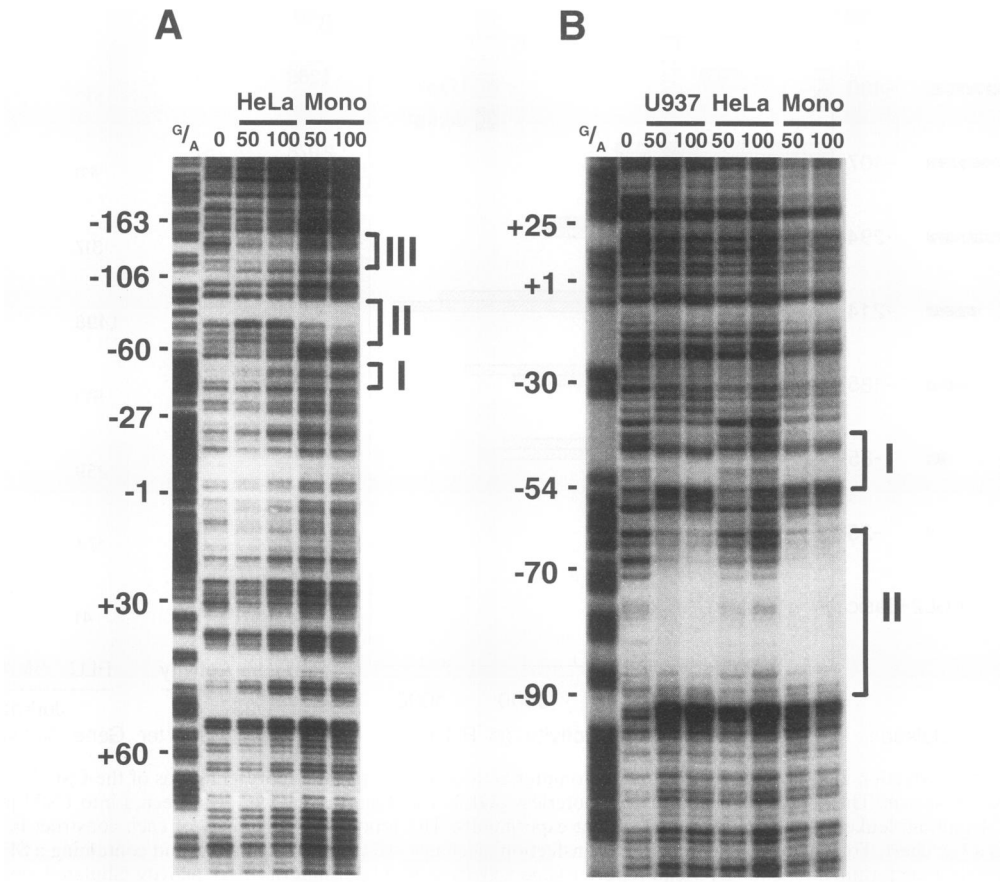


FIG. 2. DNase I footprinting of the CSF-1 receptor promoter. The CSF-1 receptor 5′ upstream DNA fragment was end labeled on the coding strand (A) or noncoding strand (B) and incubated with 0, 50, and 100 μg of nuclear protein from U937, HeLa, or Mono Mac 6 cells in the presence of 4 μg of double-stranded poly(dI-dC). Lane G/A is a marker consisting of the probe DNA subjected to G+A-specific Maxam-Gilbert cleavage. Numbers at the left side of each panel indicate the nucleotides upstream (−) or downstream (+) of the major transcription initiation site (+1). Sequences protected from DNase I digestion are indicated by brackets, and the roman numerals refer to the entire protected region. (C) CSF-1 receptor promoter sequence and three protected regions. Protected regions from the labeled coding strand and noncoding strand are indicated by brackets above and below the DNA sequence, respectively.

disappearance of a DNA band around bp −41 and the appearance of hypersensitive bands around bp −44 to −48. When noncoding-strand-labeled DNA fragments were used as probes, only the nuclear proteins from U937 and Mono Mac 6 monocytic cells, but not HeLa cells, showed strong protection in region I. A hypersensitive site at bp −47 of region I developed when noncoding-strand-labeled DNA fragments interacted with nuclear proteins from monocytic cells. Region I is bound by the monocytic cell- and B-cell-specific transcription factor PU.1 and another non-tissue-specific factor (refer-

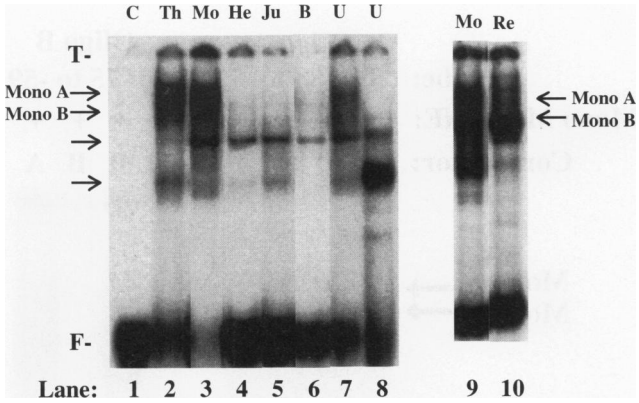


FIG. 3. EMSA of DNA-protein interactions at region II of the CSF-1 receptor promoter with nuclear proteins from different cell lines. A double-stranded CSF-1 receptor promoter oligonucleotide extending from bp −88 to −59 was end labeled and incubated in the absence of nuclear protein (lane 1) or in the presence of 10 μg of nuclear protein prepared from THP-1 (monocytic) cells (lane 2), Mono Mac 6 (monocytic) cells (lanes 3 and 9), HeLa (epithelioid) cells (lane 4), Jurkat (T-lymphoid) cells (lane 5), BJA-B (B-lymphoid) cells (lane 6), U937 (monocytic) cells (lane 7), and REX (T-lymphoid) cells (lane 10). Lanes 1 to 8 and lanes 9 and 10 are from two independent but similar experiments. A single-stranded (coding-strand) CSF-1 receptor promoter oligonucleotide extending from bp −88 to −59 was end labeled and incubated with 10 μg of nuclear protein prepared from U937 cells (lane 8). T, top of the gel; F, position of migration of the free oligonucleotide. Arrows indicate two bands shifted by tissue-specific proteins (Mono A and Mono B). The unlabeled arrows on the left side correspond to the positions of two bands shifted with single-stranded oligonucleotide.

ence 30 and data not shown). Region II was protected by nuclear proteins from Mono Mac 6 and U937. The nuclear extract from HeLa cells did not show any protection of the coding-strand-labeled DNA probe (Fig. 2A). With the noncoding-strand-labeled DNA probe, nuclear extract from HeLa cells generated a DNase I-hypersensitive band at bp −93 similar to that formed by nuclear extracts from monocytic cell lines U937 and Mono Mac 6. In addition, there was a decrease of DNase I sensitivity at bp −69 compared with DNA without any nuclear extract. Region III is protected by nuclear proteins from each type of cells. With DNase I footprinting analysis, purified Sp1 gave a strong protection in region III (data not shown).

Because the protection at region II appeared to be specific for monocytic cells, EMSA was used to further study the tissue distribution of the factor or factor(s) that bind to these sequences. A ^{32}P-labeled double-stranded oligonucleotide extending from bp −88 to −59 was used as the probe. As shown in Fig. 3, two major shifted bands were detected with nuclear proteins from monocytic THP-1, Mono Mac 6, and U937 cells but not with nuclear proteins from a number of nonmonocytic cell types, including lymphoid Jurkat (T) and BJA-B (B) leukemia cells and epithelial HeLa carcinoma cells. In these latter three nonmonocytic cell types, very faint levels of the faster-migrating complex can be observed upon overexposure of the autoradiogram. Nuclear protein from the early T-cell line REX contained significant amounts of the faster-migrating shifted band. We have named the slower-migrating complex Mono A and the faster-migrating complex Mono B. There were two faster-migrating nonspecific bands that were also evident when a single-stranded oligonucleotide was used as the probe (Fig. 3, lane 8).

Mutations in region II abolish binding of factors Mono A and Mono B to region II. To further study the interaction of monocytic nuclear proteins with region II of the CSF-1 receptor promoter, specific mutations were programmed into region II oligonucleotides and tested for the ability to compete for the Mono A- and Mono B-binding activities. Particularly informative mutant oligonucleotides are shown in Fig. 4A, together with competition analysis of mutant unlabeled oligonucleotides for binding to a wild-type region II oligonucleotide probe (Fig. 4B). Mutant A oligonucleotide competed for the binding of the Mono B complex but not the Mono A complex. By contrast, mutant B oligonucleotide competed for the Mono A complex but not for the Mono B complex. These results suggested that the slower-migrating monocyte-specific complex (Mono A) was formed by the interaction of nuclear protein with the 5′ end of the region II probe and the more rapidly migrating complex (Mono B) with the 3′ end.

To further characterize the interaction of Mono A and Mono B components with region II of the CSF-1 receptor promoter, double-stranded oligonucleotides representing the 5′ (oligonucleotide A, bp −88 to −73) and 3′ (oligonucleotide B, bp −75 to −59) sequences of region II were used in band shift competition analyses (Fig. 4C). Mono A specifically bound to 5′ region II oligonucleotide A (lanes 1 to 4). This binding was competed for by unlabeled oligonucleotide A (lane 4) but not by unlabeled oligonucleotide B (lane 3). Mono B specifically bound to 3′ region II oligonucleotide B (lanes 5 to 8).

The DNA sequence TGTGGT in oligonucleotide B matches the consensus sequence for nuclear factor PEBP2/CBF binding (5, 21, 23). To determine whether Mono B is related to the PEBP2/CBF family, EMSA was performed with the PEBP2/CBF-binding site GGATATCTGTGGTAAGCA from the Moloney murine leukemia virus enhancer as a competitor (27). As shown in Fig. 5A, the Mono B complex can be competed for efficiently with both oligonucleotide B and the PEBP2/CBF-binding site but not with the CSF-1 receptor PU.1-binding site (30). These data suggest a close relationship between Mono B and PEBP2/CBF. To further characterize Mono B, antiserum raised against the N-terminal region of one of the PEBP2/CBF family members, AML1 (7), was used in the EMSA analysis. As shown in Fig. 5B, we detected a supershifted band with AML1 antiserum but not with antiserum against nuclear factor Oct-1. These data confirmed that Mono B is a member of the PEBP2/CBF family and identical to or closely related to AML1.

Protein-DNA interactions of Mono A and Mono B with region II sites are critical for CSF-1 receptor promoter activity. To analyze the function of the protein-DNA interactions in region II, specific mutations were generated in this region of the CSF-1 receptor promoter luciferase construct pM-CSF-R-luc with the sequences shown in Fig. 4A, in the context of the −416 to +71 CSF-1 receptor promoter, to generate pM-CSF-R(MA)-luc and pM-CSF-R(MB)-luc. pM-CSF-R(MA)-luc and pM-CSF-R(MB)-luc contain mutations in the binding sites for Mono A and Mono B, respectively (Fig. 4). Results of competion experiments with mutant A and mutant B oligonucleotides (Fig. 4) indicated that mutant A no longer bound Mono A but did not affect Mono B binding, and mutant B affected only the binding of Mono B. To further confirm that these mutations did not generate any new interactions with other nuclear factors, EMSA was performed with either mutant A or mutant B as a probe (Fig. 6A). Mutant A probe only generated a Mono B complex (lane 2), which could be abolished either by mutant A or wild-type oligonucleotide (lane 4). The mutant B probe generated only a Mono A

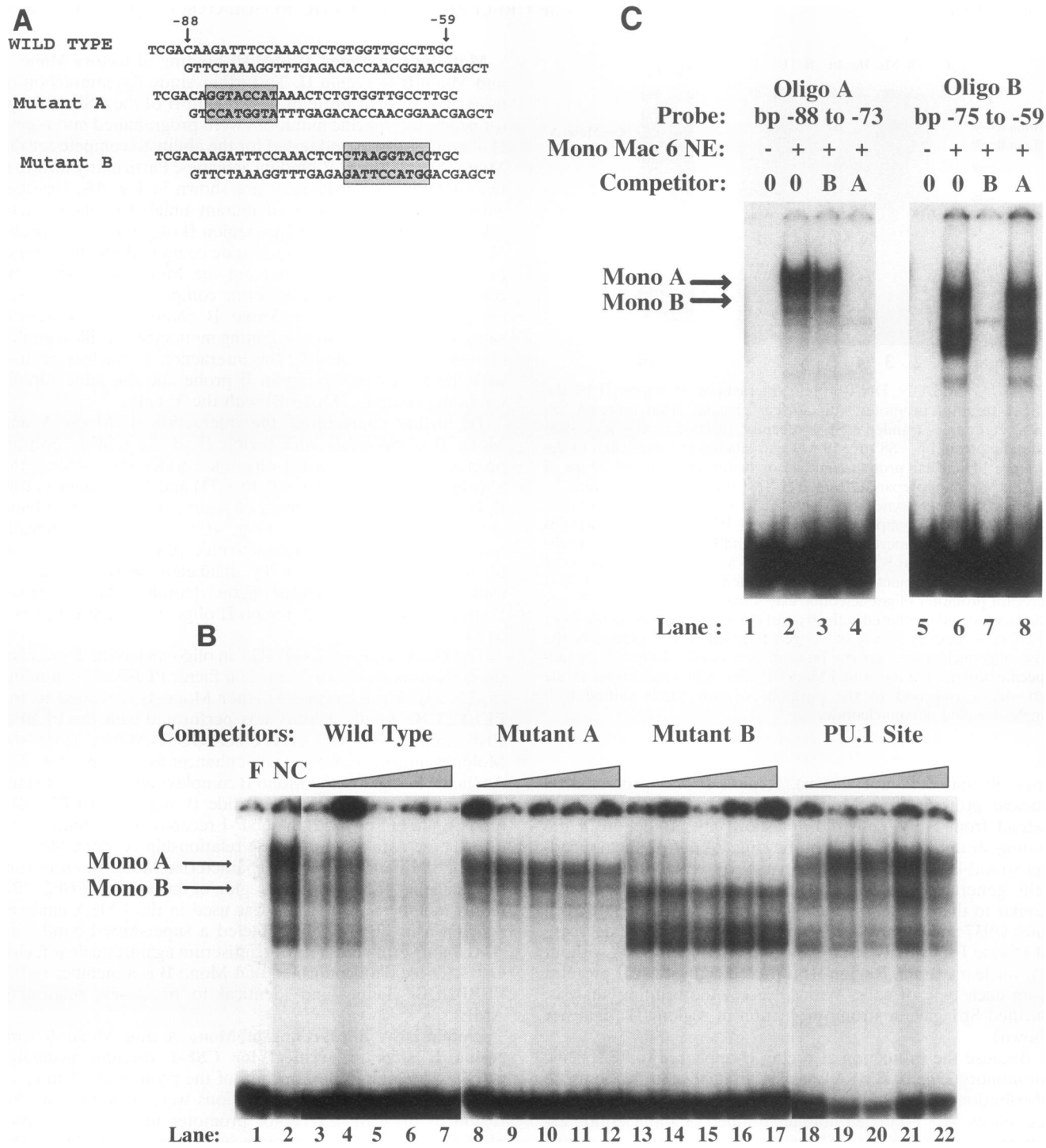


FIG. 4. EMSA analysis of region II sequences from the CSF-1 receptor promoter. (A) Sequences of CSF-1 receptor promoter bp −88 to −59 wild-type and mutant double-stranded oligonucleotides used in the band shift (panel B and Fig. 6A) and transfection (Fig. 6) experiments. The shaded boxes indicate the locations of mutations. (B) For EMSA of DNA-protein interactions including region II of the CSF-1 receptor promoter, radiolabeled wild-type double-stranded CSF-1 receptor promoter oligonucleotide was incubated with 5 μg of nuclear protein prepared from Mono Mac 6 cells. The following unlabeled, double-stranded competitor oligonucleotides were added at increasing (5-, 10-, 25-, 50-, and 100-fold) molar excess over probe oligonucleotide: lanes 3 to 7, CSF-1 receptor promoter bp −88 to −59 (wild type); lanes 8 to 12, CSF-1 receptor promoter mutant A; lanes 13 to 17, CSF-1 receptor promoter mutant B; lanes 18 to 22, CSF-1 receptor promoter bp −59 to −30 (PU.1-binding site). In lane 1, no nuclear protein was added to the binding reaction; in lane 2, 5 μg of nuclear protein from Mono Mac 6 cells was added in the absence of competitor. The arrows marked Mono A and Mono B mark the specific complexes binding to region II in monocytic cells. (C) EMSA of the interactions of the upstream (5′) and downstream (3′) sequences of region II of the CSF-1 receptor promoter with monocytic proteins. Double-stranded CSF-1 receptor promoter oligonucleotide A, extending from bp −88 to −73 (lanes 1 to 4), or oligonucleotide B, extending from bp −75 to −59 (lanes 5 to 8), was labeled and incubated in the absence (lanes 1 and 5) or presence (lanes 2 to 4 and 6 to 8) of 5 μg of nuclear protein prepared from Mono Mac 6 cells. Lanes 3 and 7 also contain a 100-fold molar excess of unlabeled oligonucleotide B in the binding reaction mixture. Lanes 4 and 8 have a 100-fold molar excess of unlabeled oligonucleotide A in the binding reaction mixture. Other symbols are as described for panel B.

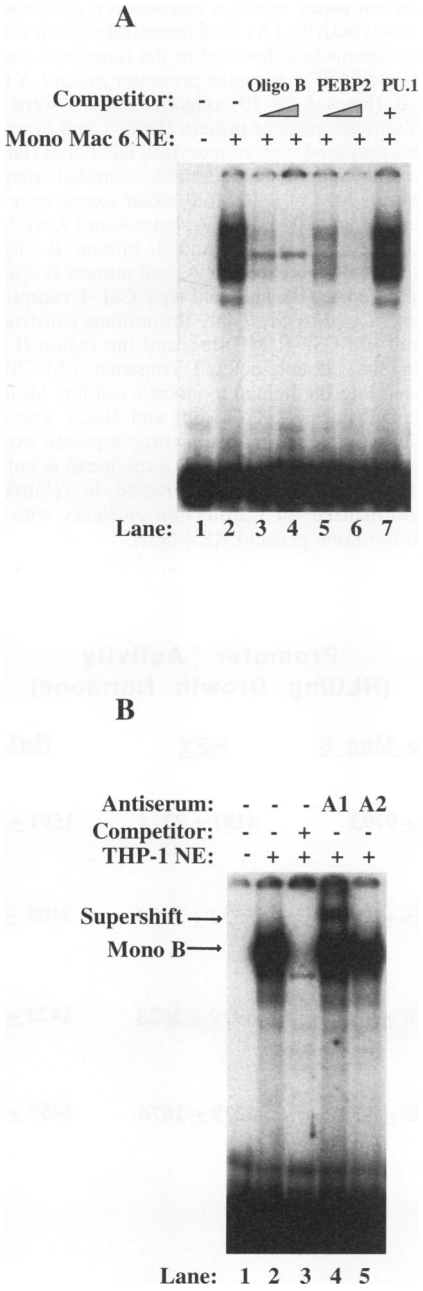


FIG. 5. Mono B is related to PEBP2/CBF. (A) A double-stranded CSF-1 receptor promoter oligonucleotide extending from bp −75 to −59 was labeled and incubated without any nuclear protein (lane 1) or with 5 μg of nuclear protein prepared from monocytic Mono Mac 6 cells (lanes 2 to 7). The following unlabeled, double-stranded competitor oligonucleotides were added: lane 2, no added competitor; lane 3, 10-fold molar excess self (oligonucleotide B) competitor; lane 4, 50-fold molar excess of self competitor; lane 5, consensus PEBP2/CBF site from the Moloney murine leukemia virus enhancer (5′ GGATATCTGTGGTAAGCA 3′) (27), 10-fold molar excess; lane 6, PEBP2/CBF competitor, 50-fold molar excess; lane 7, CSF-1 receptor PU.1 site, 50 fold molar excess. (B) The CSF-1 receptor oligonucleotide used for panel A was labeled and incubated without any nuclear protein (lane 1) or with 5 μg of nuclear protein prepared from monocytic THP-1 cells (lanes 2 to 5). In lane 3, a 100-fold molar excess of self (oligonucleotide B) competitor oligonucleotide was added. In lane 4, 1 μl of an anti-AML1 antiserum (7) was added to the extract prior to the addition of labeled probe. In lane 5, 1 μl of an anti-Oct-1 antiserum was added.

complex (lane 7), which could be abolished by either mutant B (lane 8) or wild-type (lane 9) oligonucleotide. Transient transfection experiments showed that each mutation decreased CSF-1 receptor promoter activity to 13% of the wild-type level in monocytic cells (Fig. 6B). These mutations did not affect promoter activity in nonmonocytic HeLa and REX cells, although the reporter luciferase activity was still more than 10 times higher than the promoterless luciferase construct activity (Fig. 6B). Thus, region II and its binding proteins are essential for tissue-specific CSF-1 receptor promoter activity.

As we reported previously, the myeloid cell- and B-cell-specific transcription factor PU.1 binds to the CSF-1 receptor promoter at bp −51 to −33 (region I in Fig. 2) (30). The mutation at region I abolishes PU.1 binding and decreases CSF-1 receptor promoter activity in a tissue-specific manner to 16% of wild-type promoter activity. To understand the contributions of PU.1 and proteins binding to region II to CSF-1 receptor promoter activity, deletion construct pMCSF-R(DD)-luc, containing a bp −86 to −37 deletion, was used in transient transfections. As shown in Fig. 6, this deleted CSF-1 receptor promoter construct lost almost all activity in monocytic cells. In another set of four independent experiments, a deletion from bp −86 to −62, removing the Mono A- and Mono B-binding sites, reduced promoter activity 16% ± 5% (standard deviation, comparable to that shown in Fig. 6B for either mutant A (13%) or mutant B (13%) alone. In summary, the results shown in Fig. 6 demonstrate that both region II proteins and PU.1 are important for CSF-1 receptor promoter activity.

CSF-1 receptor promoter region II functions as a tissue-specific enhancer. To further demonstrate the role of region II-specific factors for promoter activity, these sequences were multimerized and inserted upstream of a basal thymidine kinase promoter-luciferase construct, pT81-luc, to form $p(Mono)_4T81$-luc, $p(Mono)_6T81$-luc, and $p(Mono)_{6R}T81$-luc. These constructs and the parent pT81-luc construct were transiently transfected into monocytic Mono Mac 6 cells and nonmonocytic REX and HeLa cells (Fig. 7). Promoter activity increased 20- to 30-fold when multimerized region II constructs were transfected into Mono Mac 6 cells, while promoter activity in nonmonocytic cells remained at near baseline levels. In addition, when the orientation of the hexamer was reversed in $p(Mono)_{6R}TK81$-luc, region II had slightly greater ability to act as an activator in monocytic cells, indicating orientation-independent activity mediated through these sequences.

DISCUSSION

Previous studies have indicated that the monocyte/macrophage specificity of the human CSF-1 receptor is mediated by transcriptional mechanisms and that as little as 416 bp of the promoter can direct monocyte-specific expression of reporter genes in transient transfections of human cell lines (18, 30). In these studies, we identified a functional binding site for the macrophage factor PU.1, which was shown to play a critical role in macrophage-specific expression of the promoter (30).

In this report, we extended these studies to examine what other factors might regulate this promoter. Deletion studies demonstrated that as little as 85 bp of upstream sequence directed nearly threefold-higher levels of promoter activity with nearly twofold-greater relative specificity as compared with results with a larger 480-bp promoter. These findings suggest the presence of negative regulatory elements with some degree of cell type specificity and, importantly, that the major positive factors determining macrophage specificity act on a limited 85-bp region.

What transcription factors interact with this 85 bp region?

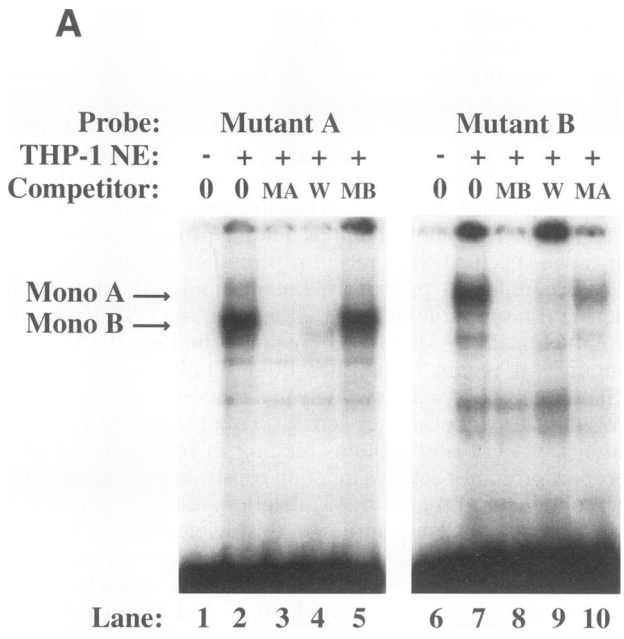
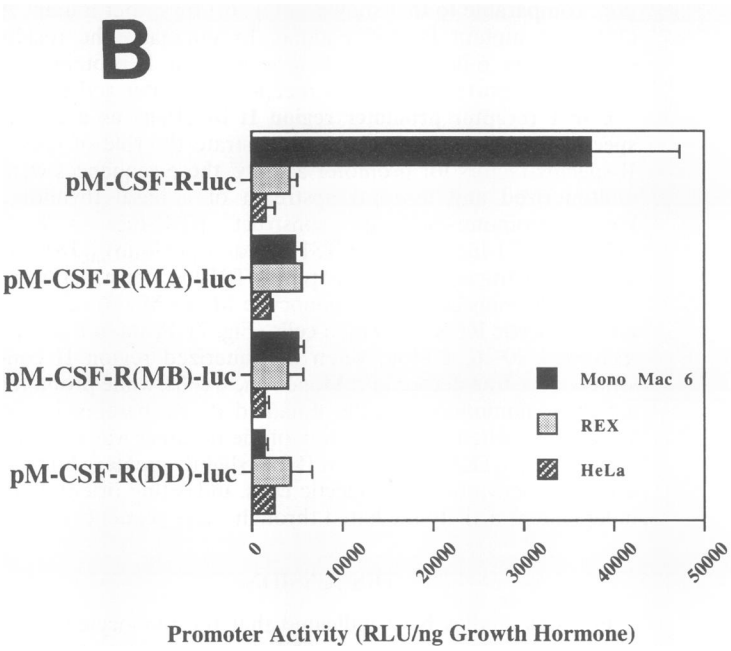


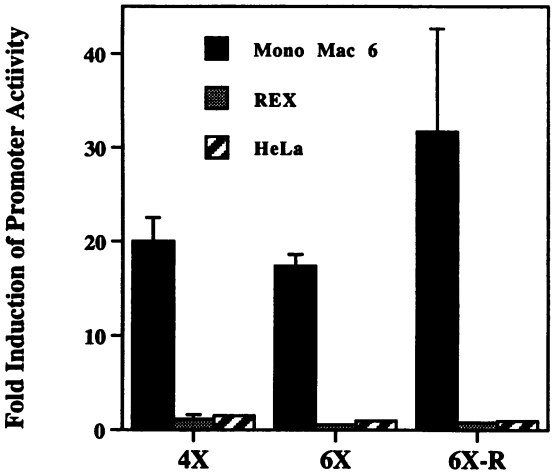
FIG. 6. Nuclear factor Mono A and Mono B are critical for CSF-1 receptor promoter activity. (A) To demonstrate which complexes bind to the mutant oligonucleotides used in the functional studies in panel B, double-stranded CSF-1 receptor promoter mutant A (lanes 1 to 5) and mutant B (lanes 6 to 10) oligonucleotides were labeled and incubated without any nuclear protein (lanes 1 and 6) or with 5 μg of nuclear protein prepared from monocytic THP-1 cells (lanes 2 to 5 and 7 to 10). The following unlabeled, double-stranded competitor oligonucleotides were added at a 100-fold molar excess over probe oligonucleotide: lanes 3 and 10, mutant A; lanes 4 and 9, wild-type bp −88 to −59 oligonucleotide; lanes 5 and 8, mutant B oligonucleotide. Sequences of the wild-type, mutant A, and mutant B oligonucleotides are shown in Fig. 4A. (B) The wild-type CSF-1 receptor promoter-luciferase construct pM-CSF-R-luc, the mutant constructs pM-CSF-R(MA)-luc and pM-CSF-R(MB)-luc, and the region II and region I (PU.1-binding site) double-deleted construct pM-CSF-R(DD)-luc were transfected into the human monocytic cell line Mono Mac 6 and nonmonocytic cell lines REX (T cell) and HeLa. The average promoter activities were generated from three separate experiments for each cell line. The standard deviation of the mean is indicated by the error bars. Luciferase activities (expressed in relative light units [RLU]) are normalized for transfection efficiency with the cotransfected growth hormone plasmid RSV-hGH.



	Promoter Activity (RLU/ng Growth Hormone)		
	Mono Mac 6	REX	HeLa
pM-CSF-R-luc	37547 ± 9793	4197 ± 2214	1591 ± 980
pM-CSF-R(MA)-luc	4845 ± 719	5533 ± 1684	2108 ± 241
pM-CSF-R(MB)-luc	5126 ± 616	3979 ± 2028	1422 ± 422
pM-CSF-R(DD)-luc	1388 ± 360	4279 ± 1676	2459 ± 145

DNase I footprint experiments revealed that besides the PU.1 site at bp −40, there is a single region (region II) bound by factors from macrophage cells. Further analysis demonstrated at least two distinct complexes in EMSA with probes corresponding to sequences from this region. Functional studies show that both play a significant role in macrophage expression of the promoter. The sequences at the 5′ and 3′ ends of region II, containing the binding sites for Mono A and Mono B, respectively, are not similar, suggesting that these two complexes contain different DNA-binding activities and are not simply different isoforms of a single DNA-binding activity.

The results of competition studies (Fig. 5A) and supershift experiments (Fig. 5B) indicate that Mono B contains a member of the PEBP2/CBF family of transcription factors. These factors consist of heterdimers between DNA-binding α subunits, which recognize the consensus site TGTGGT, and a β subunit which does not bind DNA directly but enhances the binding of the α subunit (26, 27). Multiple α subunit genes and alternatively spliced isoforms of the α and β subunits have been detected. The α subunits include a *Drosophila runt* homology domain which mediates DNA binding and protein-protein interactions. One of the human α genes (*AML1*) is located at the breakpoint of the relatively common translocation t(8;21)(q22;q22) found in one of the most common forms of FAB M2 AML. The β chain is also involved in a chromosome translocation [inv(16)(p13q22)] associated with FAB M4eo AML. Therefore, each of the two chains of the PEBP2/CBF heterodimer is directly implicated the pathogenesis of



	Mono Mac 6	REX	HeLa
$\frac{p(Mono)_4TK81\text{-}luc}{pTK81\text{-}luc}$	20.0 ± 2.4	1.5 ± 0.3	1.1 ± 0.5
$\frac{p(Mono)_6TK81\text{-}luc}{pTK81\text{-}luc}$	17.3 ± 1.2	0.5 ± 0.1	1.0 ± 0.4
$\frac{p(Mono)_{6R}TK81\text{-}luc}{pTK81\text{-}luc}$	31.7 ± 11.0	0.8 ± 0.1	1.0 ± 0.1

FIG. 7. Enhancer activity of the CSF-1 receptor promoter region II. CSF-1 receptor promoter bp −88 to −59 oligonucleotides were ligated head to tail to form a tetramer and a hexamer and inserted in front of the herpes simplex virus thymidine kinase promoter-luciferase constructs pT81-luc to form $p(Mono)_4T81$-luc, $p(Mono)_6T81$-luc, and $p(Mono)_{6R}T81$-luc as described in Materials and Methods. These constructs were transfected into monocytic Mono Mac 6 cells and nonmonocytic REX and HeLa cells. The average promoter activities were generated from three separate experiments for each cell line. Luciferase activities were normalized for transfection efficiency with the cotransfected growth hormone plasmid RSV-hGH. Results are presented as the normalized luciferase units for $p(Mono)_4T81$-luc, $p(Mono)_6T81$-luc, or $p(Mono)_{6R}T81$-luc divided by those for pT81-luc. The standard deviation of the mean is indicated by the error bars.

AML. As has been recently pointed out (11), the fusion proteins produced in these translocations could block normal myeloid differentiation by altering normal PEBP2/CBF function, which serves to activate the expression of at least two important myeloid target genes, those encoding myeloperoxidase and the CSF-1 receptor.

In our supershift studies (Fig. 5B), an antiserum raised against the 17-amino-acid N terminus of AML1 shifted only a fraction of the Mono B complex. The same results were obtained by other investigators (7, 11). This serum is likely to react with the related PEBP2αA protein and perhaps other family members as well. The unshifted Mono B complex could consist of a PEBP2/CBF α chain with an amino terminus not recognized by this antiserum, either because of sequence divergence or because the epitope is in a conformation not recognized by the antiserum. Alternatively, it is still possible that part of the unshifted Mono B complex is formed by an unrelated protein. The identity of the Mono A factor remains to be determined. Mono A can bind independently in the absence of the Mono B site, but our competition studies (Fig. 6A) show that the mutant A oligonucleotide (containing an intact Mono B site) variably and partially competes for Mono A binding, suggesting cooperativity in binding or some type of similarity between proteins of the Mono A and Mono B complexes. PEBP2/CBF has been described to interact with either the Ets-1 (28) or Myb (4) transcription factor, and the adjacent Mono A site includes a GGAA core characteristic of Ets family-binding sites. Whether Mono A represents a member of the Ets family (which includes the PU.1 factor binding downstream of region II) remains to be determined.

We found no direct evidence for interaction between PU.1 and the upstream factors. We did not detect PU.1-binding-site complexes that migrate more slowly than in vitro translated PU.1, suggesting the absence of formation of a complex

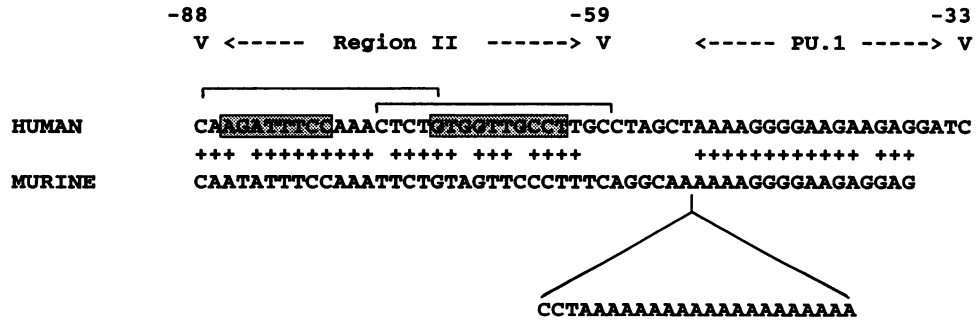


FIG. 8. Conservation of sequence similarity of the human and murine CSF-1 receptor promoters in the region II- and PU.1-binding domains. Shown on the top line are the human region II (−88 to −59) and PU.1 site (the purine core is indicated); shown on the bottom line is the murine promoter. Identities are indicated with plus signs between the lines. The murine gene contains an additional 23 bases, including a string of 20 adenine residues, between region II and the PU.1 site, which are not found in the human promoter. These bases are shown below the murine sequence to preserve the alignment. The bases in region II which were mutated to form mutant A and mutant B (Fig. 4A) are shaded, while the sequences present in the oligonucleotides which bind separately to Mono A and Mono B (Fig. 4C) are indicated with brackets above the human sequence.

between PU.1 and another factor. Furthermore, PU.1 and the upstream factors bind with strong affinity to oligonucleotides that contain only single binding sites for either PU.1 or the two upstream factors, demonstrating that the factors can bind in vitro independently of each other. Finally, mutations in the PU.1 site or the region II sites each decrease CSF-1 receptor promoter activity significantly, demonstrating that the PU.1 factor cannot replace the Mono A or Mono B complex, and vice versa, in stimulating full promoter activity. However, it is still possible that PU.1 and these upstream region binding factors interact, either through alterations in chromatin structure, through contacts with non-DNA-binding coactivators, or through direct protein-protein contacts that are not stable in gel shift assays.

To find common themes governing myeloid gene regulation, it is interesting to consider the CSF-1 receptor promoter in comparison with that of CD11b, a myeloid cell-specific integrin receptor (2, 15, 16). Both lack TATAA boxes and have a functionally important PU.1 site 20 to 40 bp upstream of the major 5′ transcription start site. However, the presence of an important PU.1 site is unlikely to be the only significant factor in CSF-1 receptor promoter specificity, because PU.1 itself is expressed at high levels in both macrophages and B cells (6). In the case of the CSF-1 receptor, the promoter has a second functionally important binding region within 40 bp upstream of the PU.1 site, which binds to two factors highly expressed in monocytic cells. These factors are not expressed at significant levels in B cells, perhaps explaining why the CSF-1 receptor is not expressed at high levels in B cells, despite the presence of high levels of PU.1. Our mutational analysis of the promoter (Fig. 6), in which mutations of either the Mono A or the Mono B site reduce promoter activity 87% in the presence of an intact PU.1 site, further supports the idea that the region II-binding factors are critical for macrophage expression of the CSF-1 receptor. To examine any possible functional relationship between Mono A and Mono B in directing CSF-1 receptor promoter activity, a construct which deleted both the Mono A- and the Mono B-binding sites simultaneously was used in transient transfections. The promoter activity of the double-deletion construct was similar to that of constructs containing either the Mono A or Mono B site mutation individually. This result indicates that Mono A and Mono B are both essential for monocyte-specific expression of the CSF-1 receptor.

The results reported here support the original observations of Roberts et al. (18), indicating that a 550-bp fragment immediately upstream of the transcribed region of the human CSF-1 receptor gene can mediate tissue-specific expression in myelomonocytic cells. Subsequent studies confirmed these results and demonstrated monocyte-specific transcriptional activity by nuclear run-on analysis (30). An entirely different model was recently proposed by Yue et al. (29), who studied a similar region of the murine CSF-1 receptor gene. They concluded that the gene contains a constitutively active promoter and that the macrophage-specific production of full-length mRNA is controlled by sequences in intron 2 that regulate transcript elongation. Whether this discrepancy reflects inherent differences between the human and murine promoters is not clear, although the striking conservation between the two promoters in the region II- and PU.1-binding domains (Fig. 8) suggests that they could direct monocyte-specific expression of both the murine and human CSF-1 receptors. The concept of the CSF-1 promoter itself as the major determinant of tissue specificity is supported by the demonstration that the upstream fragment identified by Roberts et al. (18) directs macrophage-specific expression of the receptor in transgenic mice (8). Finally, we have shown that the Mono A and Mono B protein complexes are preferentially detected in mobility shift assays in cells of the monocyte lineage and bind to sequence elements in region II of the promoter and that these sequences are required for tissue-specific expression of this promoter fragment. Thus, the preponderance of data support the conclusion that the CSF-1 promoter sequences themselves are responsible for macrophage-specific expression of the CSF-1 receptor and certainly argue against the interpretation that the CSF-1 promoter is constitutively active in all cell types. Further characterization of the region II-binding proteins should aid in elucidating the mechanism of how a small number of DNA-binding proteins within a small promoter region can mediate macrophage-specific expression.

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REFERENCES

1. **Brasier, A. R., J. E. Tate, and J. F. Habener.** 1989. Optimized use of the firefly luciferase assay as a reporter gene in mammalian cell lines. BioTechniques **7**:1116–1122.
2. **Chen, H. M., H. L. Pahl, R. J. Scheibe, D. E. Zhang, and D. G. Tenen.** 1993. The Sp1 transcription factor binds the CD11b promoter specifically in myeloid cells in vivo and is essential for myeloid-specific promoter activity. J. Biol. Chem. **268**:8230–8239.
3. **Dignam, J. D., R. M. Lebovitz, and R. G. Roeder.** 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11**:1475–1489.
4. **Hernandez-Munain, C., and M. S. Krangel.** 1994. Regulation of the T-cell receptor delta enhancer by functional cooperation between c-Myb and core-binding factors. Mol. Cell. Biol. **14**:473–483.
5. **Kamachi, Y., E. Ogawa, M. Asano, S. Ishida, Y. Murakami, M. Satake, Y. Ito, and K. Shigesada.** 1990. Purification of a mouse nuclear factor that binds to both the A and B cores of the polyomavirus enhancer. J. Virol. **64**:4808–4819.
6. **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.
7. **Meyers, S., J. R. Downing, and S. W. Hiebert.** 1993. Identification of AML-1 and the (8;21) translocation protein (AML-1/ETO) as sequence-specific DNA-binding proteins: the runt homology domain is required for DNA binding and protein-protein interactions. Mol. Cell Biol. **13**:6336–6345.
8. **Miyazaki, T., G. Suzuki, and K.-I. Yamamura.** 1993. The role of macrophages in antigen presentation and T cell tolerance. Int. Immunol. **5**:1023–1033.
9. **Miyoshi, H., K. Shimizu, T. Kozu, N. Maseki, Y. Kaneko, and M. Ohki.** 1991. t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, AML1. Proc. Natl. Acad. Sci. USA **88**:10431–10434.
10. **Nordeen, S. K.** 1988. Luciferase reporter gene vectors for analysis of promoters and enhancers. BioTechniques **6**:454–458.
11. **Nuchprayoon, I., S. Meyers, L. M. Scott, J. Suzow, S. Hiebert, and A. D. Friedman.** 1994. PEBP2/CBF, the murine homolog of the human myeloid AML1 and PEBP2β/CBFβ proto-oncoproteins, regulates the murine myeloperoxidase and neutrophil elastase genes in immature myeloid cells. Mol. Cell. Biol. **14**:5558–5568.
12. **Ogawa, M.** 1993. Differentiation and proliferation of hematopoietic stem cells. Blood **81**:2844–2853.

13. **Owen, R. D., D. M. Bortner, and M. C. Ostrowski.** 1990. *ras* oncogene activation of a VL30 transcriptional element is linked to transformation. Mol. Cell. Biol. **10:**1–9.
14. **Pahl, H. L., T. C. Burn, and D. G. Tenen.** 1991. Optimization of transient transfection into human myeloid cell lines using a luciferase reporter gene. Exp. Hematol. **19:**1038–1041.
15. **Pahl, H. L., A. G. Rosmarin, and D. G. Tenen.** 1992. Characterization of the myeloid-specific CD11b promoter. Blood **79:**865–870.
16. **Pahl, H. L., R. J. Scheibe, D. E. Zhang, H. M. Chen, D. L. Galson, R. A. Maki, and D. G. Tenen.** 1993. The proto-oncogene PU.1 regulates expression of the myeloid-specific CD11b promoter. J. Biol. Chem. **268:**5014–5020.
17. **Pevny, L., M. C. Simon, E. Robertson, W. H. Klein, S. F. Tsai, V. D'Agati, S. H. Orkin, and F. Costantini.** 1991. Erythroid differentiation in chimaeric mice blocked by a targeted mutation in the gene for transcription factor GATA-1. Nature (London) **349:**257–260.
18. **Roberts, W. M., L. H. Shapiro, R. A. Ashmun, and A. T. Look.** 1992. Transcription of the human colony-stimulating factor-1 receptor gene is regulated by separate tissue-specific promoters. Blood **79:**586–593.
19. **Sariban, E., T. Mitchell, and D. Kufe.** 1985. Expression of the c-fms proto-oncogene during human monocytic differentiation. Nature (London) **316:**64–66.
20. **Sherr, C. J.** 1990. Colony-stimulating factor-1 receptor. Blood **75:**1–12.
21. **Speck, N. A., and D. Baltimore.** 1987. Six distinct nuclear factors interact with the 75-base-pair repeat of the Moloney murine leukemia virus enhancer. Mol. Cell. Biol. **7:**1101–1110.
22. **Stanley, E. R., L. J. Guilbert, R. J. Tushinski, and S. H. Bartelmez.** 1983. CSF-1—a mononuclear phagocyte lineage-specific hemopoietic growth factor. J. Cell Biochem. **21:**151–159.
23. **Thornell, A., B. Hallberg, and T. Grundstrom.** 1988. Differential protein binding in lymphocytes to a sequence in the enhancer of the mouse retrovirus SL3-3. Mol. Cell. Biol. **8:**1625–1637.
24. **Visvader, J., and I. M. Verma.** 1989. Differential transcription of exon 1 of the human c-fms gene in placental trophoblasts and monocytes. Mol. Cell. Biol. **9:**1336–1341.
25. **Voso, M. T., T. C. Burn, G. Wulf, B. Lim, G. Leone, and D. G. Tenen.** 1994. Inhibition of hematopoiesis by competitive binding of the transcription factor PU.1. Proc. Natl. Acad. Sci. USA **91:**7932–7936.
26. **Wang, S., Q. Wang, B. E. Crute, I. N. Melnikova, S. R. Keller, and N. A. Speck.** 1993. Cloning and characterization of subunits of the T-cell receptor and murine leukemia virus enhancer core-binding factor. Mol. Cell. Biol. **13:**3324–3339.
27. **Wang, S. W., and N. A. Speck.** 1992. Purification of core-binding factor, a protein that binds the conserved core site in murine leukemia virus enhancers. Mol. Cell. Biol. **12:**89–102.
28. **Wotton, D., J. Ghysdael, S. Wang, N. A. Speck, and M. J. Owen.** 1994. Cooperative binding of Ets-1 and core binding factor to DNA. Mol. Cell Biol. **14:**840–850.
29. **Yue, X., P. Favot, T. L. Dunn, A. I. Cassady, and D. A. Hume.** 1993. Expression of mRNA encoding the macrophage colony-stimulating factor receptor (*c-fms*) is controlled by a constitutive promoter and tissue-specific transcription elongation. Mol. Cell. Biol. **13:**3191–3201.
30. **Zhang, D.-E., C. J. Hetherington, H.-M. Chen, and D. G. Tenen.** 1994. The macrophage transcription factor PU.1 directs tissue-specific expression of the macrophage colony-stimulating factor receptor. Mol. Cell. Biol. **14:**373–381.
31. **Zhang, D. E., P. R. Hoyt, and J. Papaconstantinou.** 1990. Localization of DNA protein-binding sites in the proximal and distal promoter regions of the mouse alpha-fetoprotein gene. J. Biol. Chem. **265:**3382–3391.
32. **Ziegler-Heitbrock, H. W., E. Thiel, A. Futterer, V. Herzog, A. Wirtz, and G. Riethmuller.** 1988. Establishment of a human cell line (Mono Mac 6) with characteristics of mature monocytes. Int. J. Cancer **41:**456–461.