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Cell-Specific Expression of the Macrophage Scavenger Receptor Gene Is Dependent on PU.1 and a Composite AP-1/ets Motif

KAREN S. MOULTON,[1] KATY SEMPLE,[1] HONG WU, AND CHRISTOPHER K. GLASS[2]*

Division of Cellular and Molecular Medicine[1] and Division of Endocrinology and Metabolism,[2] Department of Medicine, University of California, San Diego, La Jolla, California 92093-0656

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The type I and II scavenger receptors (SRs) are highly restricted to cells of monocyte origin and become maximally expressed during the process of monocyte-to-macrophage differentiation. In this report, we present evidence that SR genomic sequences from −245 to +46 bp relative to the major transcriptional start site were sufficient to confer preferential expression of a reporter gene to cells of monocyte and macrophage origin. This profile of expression resulted from the combinatorial actions of multiple positive and negative regulatory elements. Positive transcriptional control was primarily determined by two elements, located 181 and 46 bp upstream of the major transcriptional start site. Transcriptional control via the −181 element was mediated by PU.1/Spi-1, a macrophage and B-cell-specific transcription factor that is a member of the ets domain gene family. Intriguingly, the −181 element represented a relatively low-affinity binding site for Spi-B, a closely related member of the ets domain family that has been shown to bind with relatively high affinity to other PU.1/Spi-1 binding sites. These observations support the idea that PU.1/Spi-1 and Spi-B regulate overlapping but nonidentical sets of genes. The −46 element represented a composite binding site for a distinct set of ets domain proteins that were preferentially expressed in monocyte and macrophage cell lines and that formed ternary complexes with members of the AP-1 gene family. In concert, these observations suggest a model for how interactions between cell-specific and more generally expressed transcription factors function to dictate the appropriate temporal and cell-specific patterns of SR expression during the process of macrophage differentiation.

The development of macrophages from bone marrow precursor cells is regulated by the combinatorial actions of colony-stimulating factors, cytokines, and other hormone-like molecules. Significant progress has been made in the identification and characterization of receptors for these substances and the proximal components of the signal transduction pathways to which they are coupled. The mechanisms by which these signals are integrated at the level of transcription to coordinate proliferation and lineage-specific programs of development remain poorly understood, however.

The type I and II macrophage scavenger receptors (SRs) are representative of proteins that are highly restricted in their patterns of expression to macrophages and related cell types (18, 20, 21, 23). The two forms of the SR are derived from a single primary transcript that is alternatively spliced (15, 30). Although the physiologic roles of SRs remain uncertain, they have been proposed to function in the uptake and degradation of chemically modified proteins at sites of inflammation (9, 16). From their ability to take up and degrade oxidized low-density lipoproteins (7, 15), SRs appear to represent an important endocytic pathway that promotes the development of cholesterol-engorged macrophages that give rise to early atherosclerotic lesions (35).

SRs are expressed at low levels in circulating monocytes and become markedly upregulated during the process of monocyte-to-macrophage differentiation (6, 9). Previous studies have suggested that SR expression is regulated by macrophage colony-stimulating factor (M-CSF), which enhances the proliferation of monocyte precursor cells and is required for the survival and functional competence of mature macrophages (13, 32). SR expression is also markedly upregulated in the THP-1 monocytic leukemia cell line following induction of macrophage differentiation by the phorbol ester tetradecanoyl phorbol acetate (TPA) (1, 11, 19). Using nuclear run-on assays, we previously demonstrated that the basal transcription of the SR gene in THP-1 cells is increased approximately eightfold following TPA treatment (19).

To identify molecular mechanisms that function to regulate SR transcription, we previously isolated genomic clones containing the promoter and upstream regulatory elements of the bovine and human SR genes (19). The SR gene lacks a conventional TATA box and initiates transcription from a cluster of closely spaced start sites. Comparison of the human and bovine SR genes revealed a high degree of sequence conservation within 245 bp of the major transcriptional start site, consistent with the possibility that this region plays an important and conserved role in regulating SR transcription. In this report, we demonstrate that these sequences were sufficient to confer preferential expression of a reporter gene to cells of the monocyte/macrophage lineage. DNase I footprinting and mutational analysis of the human SR promoter region demonstrated the presence of several DNA binding activities that correlated with either positive or negative transcriptional control. Positive transcriptional control was primarily determined by two elements, located at −181 to −198 (region I) and −46 to −70 (region IV) bp from the major transcriptional start site. Positive transcriptional control of the SR promoter via the region I element was mediated by PU.1/Spi-1, a macrophage- and B-cell-specific transcription factor that belongs to the ets domain gene family (14). Positive transcriptional control through the region IV element was mediated by AP-1 family members and a distinct set of ets

* Corresponding author. Mailing address: Division of Endocrinology and Metabolism, Department of Medicine, 9500 Gilman Dr., La Jolla, CA 92093-0656. Phone: (619) 534-6011. Fax: (619) 534-8549.

domain proteins. We have recently demonstrated that these proteins include c-Jun, JunB, and ets2 (40). In concert, these results imply that the combined actions of two subclasses of ets domain proteins are essential for the preferential expression of the SR gene in cells of the monocytic lineage and further suggest a model to account for the progressive increase in SR expression that occurs during the process of monocyte-to-macrophage differentiation.

MATERIALS AND METHODS

Plasmid constructions and site-directed mutagenesis. The human SR promoter sequences from −696 to +46 and bovine SR promoter sequences from −814 to +49 were cloned upstream from the firefly luciferase cDNA in the expression vector Δ5′ PSV2 Luciferase (4). Deletions of 5′ sequences in the human SR promoter were created by PCR to amplify specific internal sequences that were then ligated into Δ5′ PSV2 Luciferase. Site-directed mutagenesis of the human SR promoter sequences was performed with a sense single-stranded template and the following oligonucleotides: I (5′-TTTCTTTTCCTTTTCAAGATCTGGTTTTTTTTAAAGC GG-3′), II (5′-TGACAAATTTAGATTGGATCCCACTGT GCATTGATG-3′), IIIa (5′-GTGCATTGATGAGCTGGAT CCGTAAACACATTAAGAAA-3′), IIIb (5′-CACATTAAG AAAGAGGGGATCCGCAGGAATGTGT-3′), IV(AP-1) (5′-TTCAACGCAGGACGGATCCCTTTCCTTTCTTCAT -3′), IV(ets) (5′-GGAATGTGTCATTTAAAGATCTCATG TACCAGATG3′), V (5′-TTCATGTACCAGAGGGATCCA TACTATGAGATAAA-3′), and VI (5′-GTAAAGAGAG AGAAAGATCTCCATCAGTGCTGCT-3′). The underlined sequences indicate the nucleotides mutated from wild-type sequences. Two independent plasmid clones containing each mutation were confirmed by dideoxy-DNA sequencing and were subcloned into Δ5′ PSV2 Luciferase.

Transfection analysis of isolated region I sequences were performed by ligating one and three copies of wild-type or mutant elements upstream from the TATA box of the rat prolactin promoter (−36 to +33) in the luciferase reporter plasmid p36B-Luciferase. The sense-strand sequences of the oligonucleotides that were subcloned in the direct repeat orientation were 5′-GATCCTTTTCACTTCTCTTTTTTTT TAAAGCGGCCTA-3′ (wild-type region I), 5′-GATCCT CACGGAGCTTTTTTTTTTAAAGCGGCCTA-3′ (mutant 2), and 5′-GATCCTTTTCACTTCTCTTTTTGGGGTAAA GCGGCCTA-3′ (mutant 5).

Cell culture and transient expression analysis. THP-1, Jurkat, P388D1, and HL-60 cells were transfected as previously described (40). In all transient transfections, cells were harvested 48 h after the time of transfection, and luciferase activity was measured in a Monolight 2010 luminometer as described previously (39). Luciferase activity was normalized to β-galactosidase activity expressed by an internal standard plasmid containing the β-actin promoter linked to the β-galactosidase gene. Transient transfection assays using HeLa, CV-1, B82, and F9 cells were performed by calcium phosphate methods (3). Transfections were performed with duplicate points at least twice. Cotransfection experiments of HeLa, F9, and CV-1 cells used 4 μg of the luciferase reporter plasmid combined with either 4 μg of a Spi-1 expression vector driven by the Rous sarcoma virus (RSV) promoter or 4 μg of an antisense Spi-1 expression vector.

Nuclear extract preparation and DNase I footprinting analysis. Nuclear extracts were prepared from THP-1, HL-60, mouse primary peritoneal macrophage, U-937, HeLa, CV-1, B82, and GC-3 cell types as previously described (40). Protein concentrations were determined by the Bradford assay. Single-end-labeled radioactive DNA probes representing either the sense or antisense strand of the SR promoter from −158 to +46 were prepared by using PCR and a pair of radioactive and nonradioactive oligonucleotides. Sense and antisense labeled probes representing the upstream human SR promoter region were similarly prepared by using a pair of oligonucleotides with their endpoints at −245 and −75 bp.

DNase I footprinting studies were performed with 10 to 20 fmol of DNA probe containing 100,000 cpm of radioactivity and 10 μl of 10% polyvinyl alcohol diluted to a final volume of 25 μl per reaction sample. The probe mixture was combined with 0 to 15 μg of nuclear extract protein diluted in 25 μl of binding buffer (100 mM KCl, 25 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.5], 12.5 mM $MgCl_2$, 1 mM dithiothreitol, 10 μM $MgSO_4$, 20% glycerol, 0.1% Nonidet P-40) and incubated at 4°C for 15 min. Limited DNase I digestions were performed at room temperature after adding 50 μl of a solution containing 10 mM $MgCl_2$ and 5 mM $CaCl_2$ and then 2 to 20 ng of DNase I (Worthington DRFF grade). After 1 min, the digestion was stopped by adding 90 μl of a solution containing 20 mM EDTA (pH 8.0), 1% sodium dodecyl sulfate, 0.2 M NaCl, and 125 μg of tRNA per ml. Samples were digested with proteinase K, extracted twice with phenol-chloroform, precipitated, and resolved on a denaturing 6% polyacrylamide gel. The patterns of DNase I footprints were visualized with Fuji Rx film.

Electrophoretic mobility shift assays. Protein-DNA complexes were detected on the basis of alterations in electrophoretic mobility (8). In all reactions, the final salt concentration was adjusted to 150 mM KCl. The DNA probes representing wild-type and mutated sequences of region I used for the sequence specificity data shown in Fig. 6 were prepared by annealing the wild-type sense-strand template, 5′-gatcggatc cTCACTTCTCTTTTTTTTTAAAGCGGCctagatctcgagggc cg-3′, with a short primer oligonucleotide, 5′-cggccctcgagatcta 3′. The uppercase letters indicate the native sequences found in the human SR promoter region. The complementary antisense strand was synthesized by adding deoxynucleotides (dATP, dGTP, dTTP, dCTP, and [α-^{32}P]dCTP) and the Klenow fragment of DNA polymerase. The sequences of the mutated sense-strand templates are listed in Fig. 5. The electrophoretic mobility shift experiments demonstrating PU.1/Spi-1 binding to region I in the SR promoter (Fig. 7) used the following oligonucleotides annealed to their respective complementary oligonucleotides: SR region I (5′-gatcTGTT TCTTTTCCTTTTCACTTCTCTTTTTg-3′), idealized PU.1 site (5′-gatccTTTCCACTTCCGCTTCCTTTTTAAAGCGG CCTagatc-3′), and immunoglobulin kappa 3′ enhancer PU.1 (5′-gatctAGGTTCTGTTTTCAGTTCCTCAAAGGgatc-3′ (24).

A rabbit polyclonal antibody recognizing the amino terminus of PU.1 and the PU.1 cDNA plasmid was provided by Richard Maki. PU.1 protein was prepared by in vitro transcription and translations procedures previously described (14). A guinea pig antiserum specific for the DNA binding domain of PU.1/Spi-1 was prepared against a purified, recombinant protein corresponding to amino acids 157 to 272.

Expression screening. A human bone marrow lambda gt11 library (Clontech) was screened for fusion proteins that recognized the minimal sequence motif of the region I element according to methods previously described (34, 36). Probes were prepared from sense and antisense oligonucleotides, containing the respective wild-type sequences 5′-CGGCT CACTTCTCTTTTT-3′ and 5′-GCCGAAAAAGAGAAG TGA-3′, that were phosphorylated with [^{32}P]ATP, annealed,

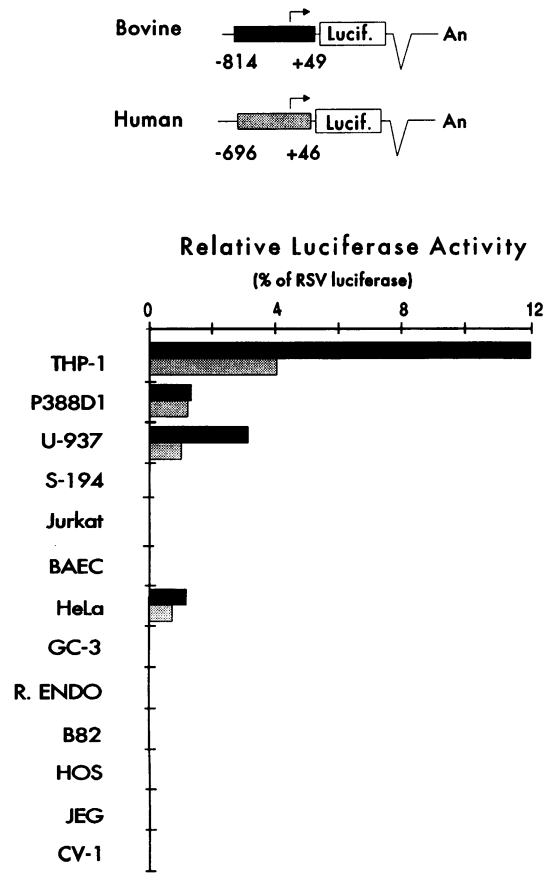

FIG. 1. The human and bovine SR promoters are preferentially expressed in cells of the monocyte/macrophage lineage. The bovine SR genomic sequence corresponding to −814 to +49 bp from the major transcriptional start site or the human SR genomic sequence from −696 to +46 were fused to a firefly luciferase cDNA, which served as a reporter gene. The resulting plasmids were transfected into the indicated cell types and harvested 48 h later for determination of luciferase activity. For each cell type, an RSV-luciferase plasmid was transfected as an external standard. The activities of the bovine (black) and human (stippled) promoter activities are expressed as a percentage of the activities determined for RSV-luciferase. The results represent the averages of at least two independent experiments for each cell type. The cell types used were THP-1 (monocytic leukemia), P388D (macrophages), U-937, (histocytic leukemia), S194 (B cells), Jurkat (T cells), BAEC (bovine aortic endothelial cells), HeLa (cervical carcinoma), GC-3 (anterior pituitary cells), R. ENDO (rabbit endothelial cells), B82 (fibroblasts), HOS (human osteosarcoma), JEG (placental cells), and CV-1 (kidney epithelium).

and ligated to high molecular weight. Positive clones were plaque purified and then tested for the absence of binding to a similarly prepared probe containing mutated region I sequences (Fig. 7A).

RESULTS

To identify regulatory factors necessary for cell-type-specific expression of the SR, human and bovine promoter sequences extending from −696 to +46 and −814 to +49 relative to the major transcriptional start sites were tested for the ability to direct the expression of luciferase activity in transient transfection assays (Fig. 1). Transfection experiments were performed in multiple monocytic, macrophage, and nonmyeloid cell types. The activities of the SR promoters were normalized to the luciferase activities of the RSV (Fig. 1) or the β-actin (data not shown) promoter to correct for different transfection efficiencies observed among the various cell types. As shown in Fig. 1, the human and bovine SR promoters functioned preferentially in THP-1, P388D1, and U-937 cells, which represent monocyte/macrophage-like cells. Significant levels of expression were also observed in HeLa cells but not in the other nonmyeloid cell lines tested. These results indicated that the SR sequences corresponding to −696 to +46 of the human gene and −814 to +49 of the bovine gene contained sufficient information to direct preferential expression of a reporter gene to monocyte/and macrophage-like cells.

A 5′ deletion analysis of the human SR promoter indicated that truncation to −245 bp from the transcriptional start site had relatively little effect on reporter gene expression (data not shown). Further deletion to −158 bp resulted in a loss of nearly two-thirds of the activity observed for the −696 construct. Subsequent deletions of the promoter from −73 to −57 bp nearly abolished the remaining promoter activity. These results suggested that sequences important for positive regulation of SR promoter activity were contained in the regions between −245 to −158 and −73 to −57. To identify DNA binding activities capable of recognizing SR promoter sequences, DNase I footprinting studies were performed. Because of the difficulty of examining this entire region with probes prepared from a single DNA fragment, separate probes corresponding to the proximal and distal regions of this sequence were used. Figure 2 illustrates the results of footprinting experiments examining the proximal region of the SR promoter from −158 to +46 bp. Nuclear extracts were prepared from unstimulated THP-1 cells, THP-1 cells treated with TPA for 16 h to induce macrophage differentiation, and P388D1 macrophages. Each of these nuclear extracts produced five footprints, labeled II through VI. The DNA binding activity recognizing region IV correlated with macrophage differentiation in that higher levels were present in P388D1 macrophages and TPA-treated THP-1 cells than untreated THP-1 cells. In contrast, the relative pattern of binding activity recognizing footprint VI was inversely correlated with macrophage differentiation, with the highest levels observed in untreated THP-1 cells. Figure 3A illustrates the results of a DNase I footprinting experiment examining DNA binding activities for the region between −245 and −122 bp. Nuclear extracts prepared from THP-1 cells, P388D1 cells, and primary mouse peritoneal macrophages produced a footprint, termed region I, extending from −181 to −198 bp, as well as a hypersensitive site at approximately −189.

Two of the footprints identified by these experiments (regions I and IV) correlated with sequences demonstrated to be important for SR promoter function in the 5′ deletion analysis. These regions were examined in further detail to determine the relative abundance of the corresponding DNA binding activities in monocyte/macrophage-like cells compared with nonmyeloid cells. As illustrated in Fig. 3A, the majority of nonmyeloid cells examined produced either no detectable footprint over region I (HeLa, GC-3, and CV-1 cells) or a partial footprint with different margins that lacked the hypersensitive site (Jurkat and B82 cells). As illustrated in Fig. 3B, DNA binding activities for region IV were most abundant in monocyte/macrophage-like cells, but incomplete footprints were also present in several other cell types that primarily involved the 5′ end of region IV. Inspection of the region IV sequence revealed it to represent a composite binding site for members of the AP-1 and ets domain families of transcription factors (see Fig. 9C). The upstream half of this binding site,

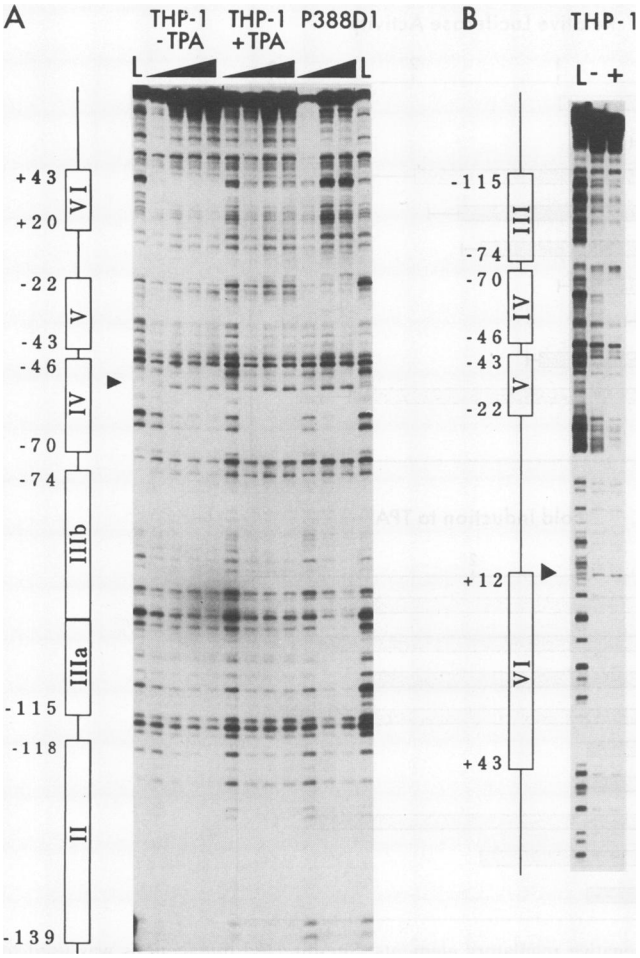

FIG. 2. DNase I footprinting analysis of the proximal region of the human SR promoter demonstrates the presence of multiple DNA binding activities. The proximal region of the human SR promoter extending from −158 to +46 was selectively labeled at either the sense (A) or antisense (B) strand. Nuclear extracts obtained from control THP-1 cells, THP-1 cells treated with TPA for 16 h, or nuclear extracts derived from the mouse P388D1 macrophage cell line were incubated at increasing concentrations (5, 10, and 15 μg/50 μl) with the radiolabeled DNA probes (L). The protein-DNA complexes were subsequently subjected to DNase I digestion and analyzed by electrophoresis through denaturing polyacrylamide gels. Footprints are indicated to the left of each footprinting ladder and are labeled II through VI. The numbers indicate the borders of the footprints as determined by comparison with a sequencing ladder. The arrowhead indicates a hypersensitive site that occurs within region IV.

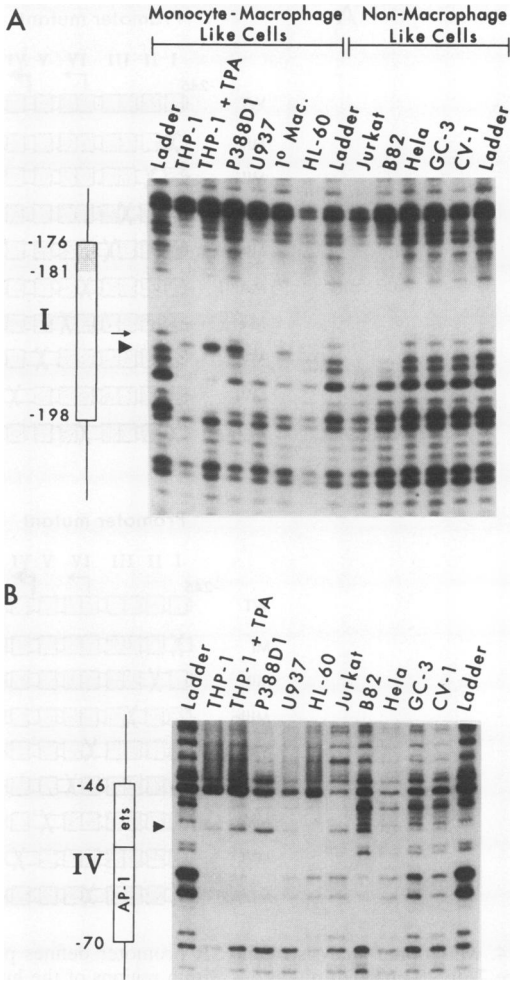

FIG. 3. Nuclear proteins that bind to regions I and IV of the SR promoter are preferentially expressed in monocyte/macrophage-like cells. (A) DNase I footprinting of the sense strand of the region from −245 to −75 of the human SR gene. Only the relevant region of the footprinting ladder is shown. A footprint spanning the region from −181 to −198 is observed with nuclear extracts obtained from monocyte/macrophage-like cells but not with extracts from non-macrophage-like cells. A slightly larger footprint, extending from −176 to −198, is observed for THP-1 extracts. A hypersensitive site, indicated by an arrowhead, is observed selectively in monocyte/macrophage-like cells. This hypersensitive site is distinct from a cleavage site (indicated by an arrow) that is abolished by monocyte/macrophage extracts but not by extracts prepared from non-macrophage-like cells. (B) Nuclear proteins that bind to the 3′ end of region IV are preferentially expressed in monocyte/macrophage-like cells. The 5′ end of the region IV footprint corresponds to an AP-1 motif, while the 3′ end represents a core binding motif for ets domain proteins. DNase I footprinting experiments were carried out with a probe corresponding to the sense strand of the human SR promoter. The arrowhead indicates a hypersensitive site within the ets recognition motif. Only the region of the gel surrounding footprint IV is illustrated.

which was recognized by DNA binding activities present in both monocyte/macrophage-like cells and nonmyeloid cells (Fig. 3B), corresponds to a nonconsensus AP-1 recognition motif. The 3′ end of region IV, which was primarily footprinted by DNA binding activities present in cells of monocytic origin and Jurkat cells, corresponds to the ets domain recognition motif.

To determine the relative importance of each footprinted region in SR promoter activity, site-directed mutagenesis was used to introduce clustered point mutations into each binding site. As illustrated in Fig. 4A, selected mutations of region I and the ets recognition motif of region IV each resulted in an approximate 50% reduction in promoter activity. In contrast, introduction of point mutations into the footprints corresponding to regions II, III, and VI resulted in 1.5- to 2.5-fold increases in promoter activity compared with the wild-type SR promoter. These observations suggested that the sequences corresponding to regions II, III, and VI were recognized by factors that functioned to inhibit SR transcription. Although a

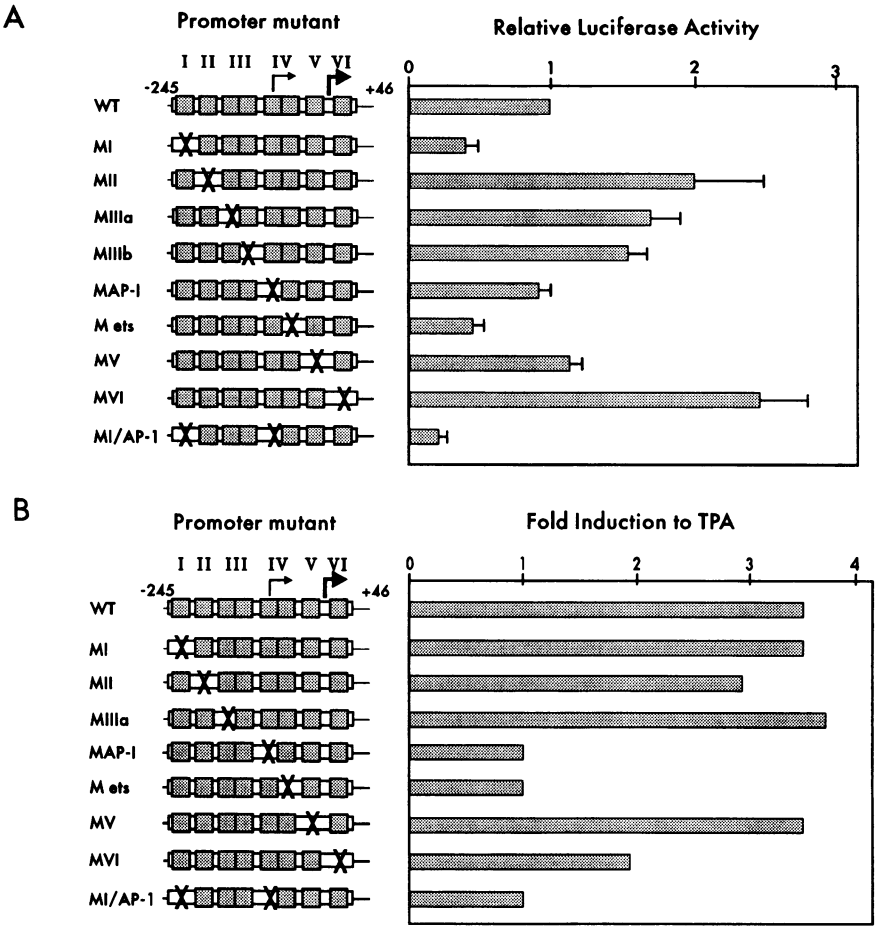

FIG. 4. Mutational analysis of the SR promoter defines positive and negative regulatory elements. Site-directed mutagenesis was used to introduce 7- to 10-bp point mutations within regions of the human SR promoter demonstrated to be recognized by nuclear proteins present in monocyte/macrophage-derived cell lines. The resulting mutant promoters were subcloned into a luciferase expression plasmid as illustrated in Fig. 1. (A) Effects of mutations on basal promoter activity. Wild-type and mutant promoter were transfected into THP-1 cells, and cells were harvested for analysis of luciferase activity 48 h later. The results are expressed as a percentage of the activity observed for the wild-type (WT) human SR promoter. (B) Effects of mutations on TPA-dependent promoter activity. Wild-type and mutant promoters were transfected into THP-1 cells. The transfected pool of cells was split for treatment with either TPA or solvent. Cells were harvested for luciferase activity 16 h following treatment. Results are expressed as the ratio of luciferase activities for TPA-treated versus untreated cells and are representative of three independent experiments.

mutation of the near-consensus AP-1 site in region IV had a minor effect alone in THP-1 cells, a double mutation targeting both region I and the AP-1 site of region IV resulted in the loss of approximately 85% of wild-type promoter activity. In contrast, selective mutation of the AP-1 site nearly abolished SR promoter function in HeLa cells, providing an explanation for the activity of the wild-type promoter in these cells (data not shown).

We next examined the effects of these mutations on the ability of the SR promoter to respond to TPA (Fig. 4B). The wild-type SR promoter conferred a threefold transcriptional response to TPA. This response was significantly less than that observed for the endogenous SR gene (19), suggesting that the sequence from −245 to +43 was insufficient to mediate the full transcriptional response to TPA. Indeed, we recently identified a TPA-dependent enhancer located 4.1 kb upstream that cooperates with the SR promoter to direct a 15- to 20-fold transcriptional response (40). In agreement with our previous studies, mutation of either the AP-1 or ets motif abolished the promoter's threefold response to TPA. In addition, mutation of the inhibitory element corresponding to region VI also resulted in a reduced fold of induction to TPA.

From the mutational analysis of the SR promoter and the results obtained from DNase I footprinting experiments, the DNA binding activity recognizing the region I sequence appeared to represent a monocyte/macrophage-specific transcription factor that played a quantitatively important role in SR promoter function. Computer-assisted comparison of the region I sequence to consensus or near-consensus binding sites for known transcription factors failed to identify a specific candidate that was likely to account for this activity. An electrophoretic mobility shift assay was therefore established to characterize the sequence requirements for high-affinity DNA binding and transcriptional activity. As illustrated in Fig. 5B and C, incubation of a radiolabeled oligonucleotide probe containing the region I footprint with nuclear extracts prepared from THP-1 cells or P388D1 macrophages resulted in the formation of several protein-DNA complexes, labeled Ia, Ib, Ic, and Id. When compared directly, the Ia and Ic complexes obtained by using THP-1 nuclear extracts ran at posi-

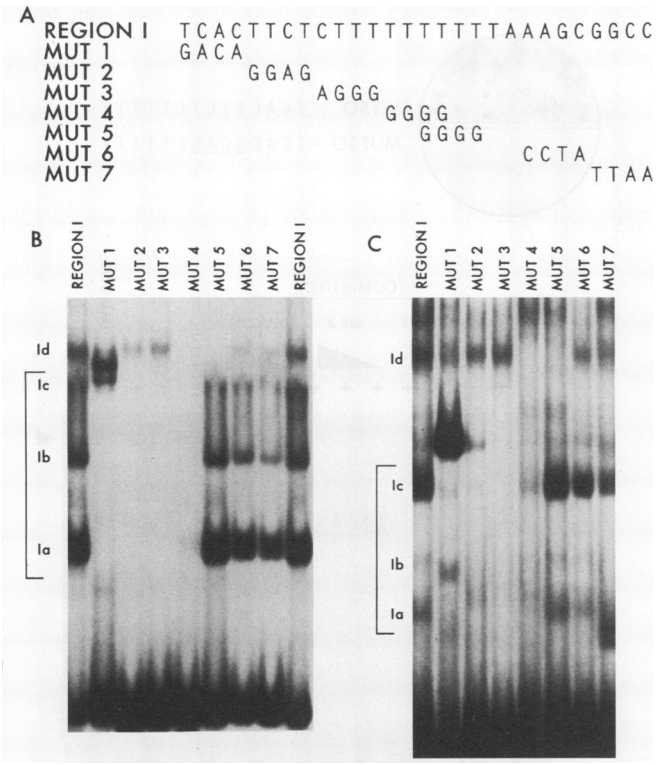

FIG. 5. Sequence specificities of DNA binding proteins that recognize the region I footprint. (A) Wild-type and mutant oligonucleotides corresponding to the region I footprint used in electrophoretic mobility shift assays. Region I indicates the sequence of the wild-type SR promoter from −198 to −171. MUT 1 through MUT 7 indicate the base pair substitutions introduced into the sequence. (B) The wild-type and mutant double-stranded oligonucleotides were radiolabeled with [γ-^{32}P]ATP and incubated with nuclear extracts derived from THP-1 cells. Four discrete complexes, labeled Ia, Ib, Ic, and Id, were observed to form on the wild-type oligonucleotide. The sequence-specific DNA binding properties of complexes Ia, Ib, and Ic could not be discriminated from each other but were distinct from the DNA binding properties of complex Id. (C) DNA binding properties of nuclear proteins obtained from P388D1 macrophages on wild-type and mutant region I oligonucleotides.

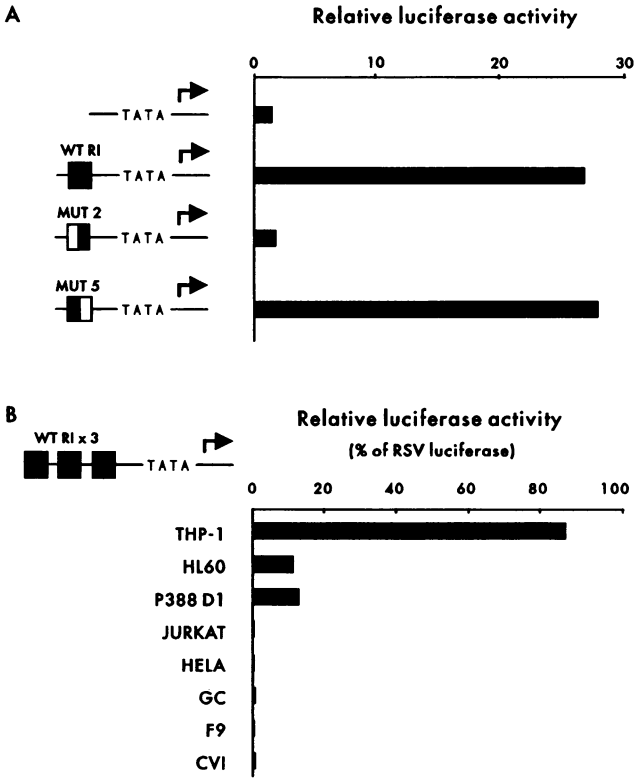

FIG. 6. Region I of the SR promoter functions as a cell-specific enhancer element. (A) A single copy of the double-stranded oligonucleotide corresponding to the wild-type region I (WT RI) element was introduced upstream of a minimal promoter derived from the rat prolactin gene (shown as TATA). This composite transcription unit was used to drive the expression of a luciferase reporter gene. Sequence requirements for region I activity were examined by introducing single copies of the oligonucleotides corresponding to mutants (MUT) 2 or 5 (illustrated in Fig. 6) upstream of the same minimal prolactin promoter. Each construct was transfected into THP-1 cells along with a β-actin/β-galactosidase plasmid, which served as an internal standard. The results are expressed as the ratio of luciferase to β-galactosidase activities. (B) Three copies of the region I sequence were introduced upstream of the minimal rat prolactin promoter and transfected into the indicated cell types. Relative transcriptional activities were determined as described for Fig. 1.

tions nearly identical to those of the Ia and Ic complexes obtained by using P388D1 nuclear extracts, suggesting that they represented corresponding proteins (data not shown). In contrast, P388D1 complex Id migrated more slowly than THP-1 complex Id.

The sequence requirements for the binding of each complex were determined by synthesizing a series of double-stranded oligonucleotides containing mutations in the region I sequence, as illustrated in Fig. 5A. Each oligonucleotide was radiolabeled and tested for the ability to be recognized by the proteins corresponding to Ia through Id. As judged from these experiments, complexes Ia, Ib, and Ic had indistinguishable DNA binding activities that overlapped but could be distinguished from the recognition sequence for complex Id (Fig. 5B). In addition, the binding of complex Id and complex Ia, Ib, or Ic appeared to be mutually exclusive in that no more-slowly migrating complex, potentially representing the binding of both classes of proteins to a single oligonucleotide, was observed. Extracts prepared from THP-1 cells were noted to primarily contain complex Ia, while extracts prepared from P388D1 cells primarily contained complex Ic.

To correlate the DNA binding activities identified by electrophoretic mobility gel shift experiments with the sequence requirements for transcriptional activity, oligonucleotides corresponding to the wild-type, mutant 2, and mutant 5 versions of region I were introduced proximal to a minimal promoter derived from the rat prolactin gene (Fig. 6A). The wild-type region I sequence functioned as an effective enhancer element in this context, directing a level of reporter expression that was approximately one-fourth of that observed for RSV-luciferase. This enhancer function was abolished by mutant 2 but not by mutant 5. These results suggested that the DNA binding activities corresponding to complexes Ia, Ib, and Ic accounted for the transcriptional activity of the region I element and that the DNA binding activity corresponding to complex Id was not required for this effect. To further examine whether the region I sequence preferentially functioned to enhance transcription in monocyte/macrophage-like cells, transfection experiments were performed in several monocyte/macrophage and nonmyeloid cell types. In addition to its activity in THP-1 cells, the

region I element was observed to enhance promoter function in P388D1 and HL-60 cells. No activity was observed in HeLa, CV-1, Jurkat, or F9 cells. These results are consistent with the observation that the region I DNA binding activity observed in DNase I footprinting assays was restricted to monocyte/macrophage-like cells.

On the basis of these observations, an expression cloning strategy was used to isolate cDNA clones encoding proteins that specifically bound to the region I sequence. Double-stranded oligonucleotides containing the minimal sequence information required for binding of complexes Ia, Ib, and Ic were phosphorylated with [γ-^{32}P]ATP, ligated to high molecular weight, and used to probe lambda gt11 expression libraries. Three independent clones were identified from screening 10^6 plaques derived from a human bone marrow cDNA library. Following plaque purification, each clone was tested for its ability to direct the expression of fusion proteins capable of binding to the region I screening oligonucleotide but not to an oligonucleotide containing a 4-bp mutation. All three clones satisfied this criteria, one of which is shown in Fig. 7A. Sequence analysis revealed that all three clones encoded Spi-1, the human homolog of PU.1, originally identified as a murine macrophage- and B-cell-specific transcription factor (14, 27). The clones contained slightly different 5′ ends, indicating that they had been independently derived.

The isolation of clones encoding Spi-1 was somewhat unexpected because the majority of previously characterized Spi-1/PU.1 binding sites contain the consensus recognition sequence GGAA, which was not present in either strand of the region I sequence. We therefore examined whether the Ia, Ib, and Ic binding activities present in THP-1 and P388D1 cells corresponded to Spi-1 and PU.1, respectively. Competition experiments were performed with the wild-type region I element, a PU.1 binding site present in the immunoglobulin kappa 3′ enhancer (24), and an idealized PU.1 element based on a consensus sequence derived from alignment of previously characterized PU.1 binding sites (37). Each of these binding sites served as an effective competitor for the formation of complexes Ia, Ib, and Ic on a wild-type region I probe (Fig. 7B). The SR region I sequence and the PU.1 site from the immunoglobulin kappa 3′ enhancer exhibited nearly equivalent competition profiles, while the consensus PU.1 binding site was somewhat more effective. In vitro-translated PU.1 bound to the SR region I element with high affinity and migrated at the same position as complex Ic. An antibody directed against the amino terminus of PU.1 specifically supershifted complex Ic but not complex Ia or Ib. These results indicate that complex Ic in P388D1 cells represented full-length PU.1.

To determine whether complexes Ia and Ib represented distinct proteins or were truncated forms of PU.1 or Spi-1, a polyclonal antiserum was raised against the PU.1/Spi-1 DNA binding domain. This antiserum blocked the binding of in vitro-translated PU.1 to the region I element and quantitatively inhibited the binding of complexes Ia, Ib, and Ic in both THP-1 (Fig. 8) and P388D1 (data not shown) cells. These results suggested that complexes Ia and Ib represented truncated forms of PU.1/Spi-1, probably due to proteolysis. The possibility was also considered that complexes Ia and Ib correspond to related members of the ets domain family that cross-reacted with the anti PU.1/Spi-1 antiserum. In particular, the ets domain protein Spi-B, which is highly related to PU.1/Spi-1 in its DNA binding domain, has generally been found to be coexpressed with PU.1/Spi-1 and to bind with high affinity to PU.1 recognition sequences (26). The DNA binding properties of in vitro-translated Spi-B were therefore com-

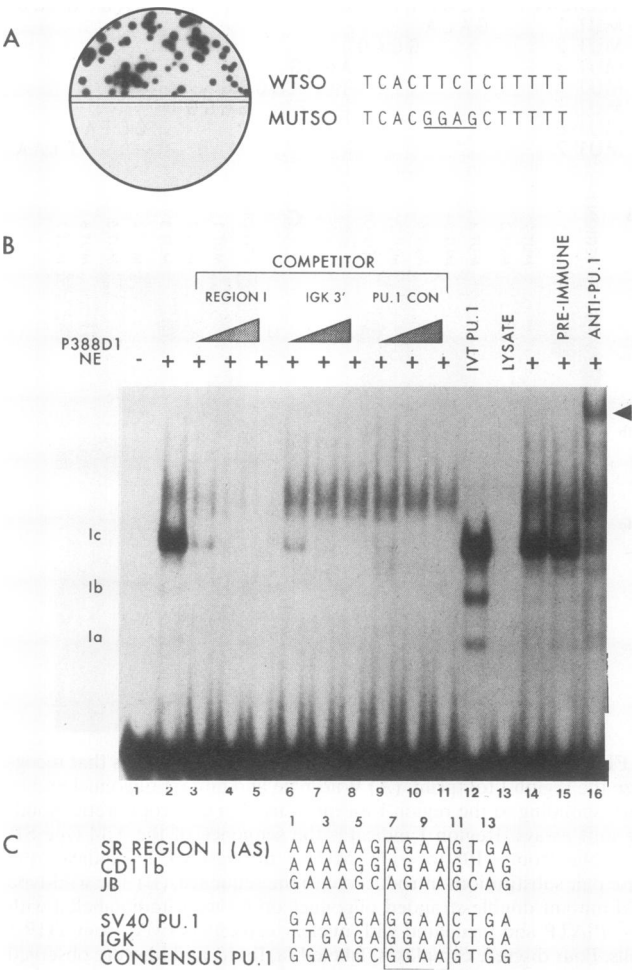

FIG. 7. The major region I DNA-binding protein corresponds to PU.1/Spi-1. (A) Cloning of cDNAs encoding proteins that bind specifically to the region I sequence. A double-stranded oligonucleotide probe corresponding to region I (WTSO) was radiolabeled with [γ-^{32}P]ATP, ligated to high molecular weight, and used to screen a human bone marrow cDNA library for fusion proteins capable of recognizing this sequence. Three positive clones were identified and plaque purified. Each clone was then tested for its ability to bind to the wild-type region I sequence (WTSO) or the indicated region I mutant (MUTSO) under stringent conditions. All three clones exhibited similar DNA binding properties, one of which is illustrated. The upper semicircle represents the hybridization pattern for the wild type, while the lower semicircle represents the hybridization pattern for the mutant. (B) Competition analysis, binding of in vitro translated PU.1, and the effects of an antibody to PU.1 indicate that complex Ic derived from P388D1 macrophages contains PU.1. Lanes 1 to 11 illustrate competition analysis of Spi-1/PU.1 binding sites for the binding of P388D1 nuclear proteins to the SR promoter region I binding site. P388D1 nuclear proteins were incubated with the region I probe in the absence or presence of 10, 200, and 2000 fmol of competing unlabeled oligonucleotide, as indicated. Lanes 12 and 13 represent the binding activities of in vitro-translated PU.1 (IVT PU.1) and control reticulocyte lysate (Lysate), respectively. Lane 15 illustrates the effects of an antibody directed against the PU.1 amino terminus. Lane 16 represents an equivalent amount of preimmune serum. (C) Comparison of the region I sequence to previously characterized binding sites for Spi-1/PU.1. The antisense (AS) strand of the region I sequence is shown. The core consensus sequence recognized by Spi-1/PU.1 is closed by a box. NE, nuclear extract; IgK, immunoglobulin kappa; CON, consensus; SV40, simian virus 40.

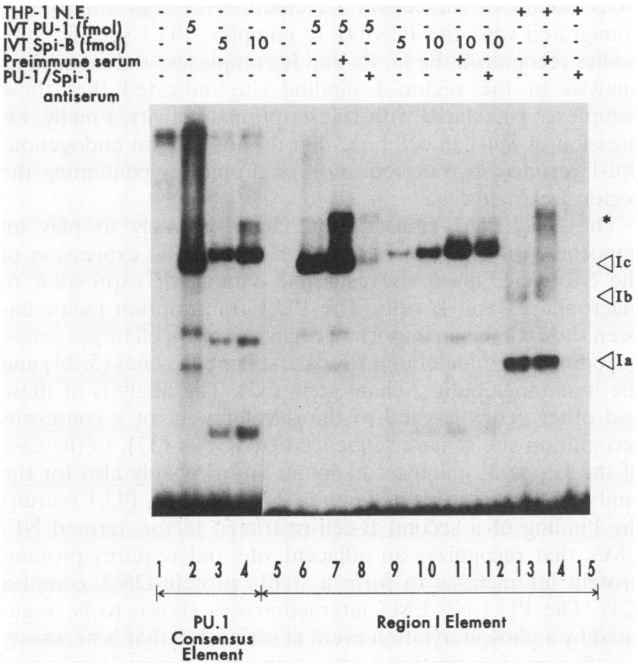

FIG. 8. The region I element distinguishes the DNA binding activities of PU.1 and Spi-B. Electrophoretic mobility shift assays were performed with in vitro-translated (IVT) PU.1, Spi-B, or THP-1 nuclear extracts (N.E.), as indicated. The amounts of PU.1 and Spi-B used in each binding reaction were quantitated by trichloroacetic acid precipitation of ^{35}S-labeled proteins as previously described (14). DNA binding reactions were performed with either a radiolabeled consensus PU.1 recognition element (lanes 1 to 4) or a minimal version of the region I element from the SR promoter that does not bind complex Id (lanes 5 to 15). Proteins were preincubated with either 1 μl of preimmune serum or 1 μl of an antiserum raised against the DNA binding domain of PU.1/Spi-1 prior to addition of the DNA probe. Preimmune serum increased the binding of PU.1 and Spi-B, as well as that of a nonspecific protein-DNA complex (indicated by an asterisk). The PU.1 antiserum quantitatively inhibited the binding of in vitro-translated PU.1 and THP-1 complexes Ia, Ib, and Ic but did not affect the binding of Spi-B.

pared with those of PU.1. Spi-B/DNA complexes could be distinguished from PU.1/DNA complexes on the basis of a slightly slower pattern of migration (Fig. 8). Intriguingly, while Spi-B bound to the consensus PU.1 recognition sequence with an affinity similar to that of PU.1, it bound to the SR region I element with an affinity that was 10- to 20-fold lower than that of PU.1 (Fig. 8 [compare lanes 6 and 10] and data not shown). These results indicated that PU.1 and Spi-B have divergent sequence preferences for high-affinity binding that permit their discrimination by the region I element. Incubation of in vitro-translated Spi-B with the PU.1/Spi-1 antiserum revealed no evidence for cross-reactivity, indicating that this antiserum was highly specific for the PU.1/Spi-1 DNA binding domain (Fig. 8). In concert, these observations exclude Spi-B as being involved in regulating SR expression through the region I element.

To confirm that Spi-1/PU.1 could activate transcription from the region I sequence, transient transfection experiments were performed in cells that lack endogenous Spi-1/PU.1. As illustrated in Fig. 9A, expression of Spi-1 in HeLa cells resulted in transcriptional activation of the minimal prolactin promoter containing the wild-type region I element. The sequence requirements for Spi-1-dependent transcription were identical

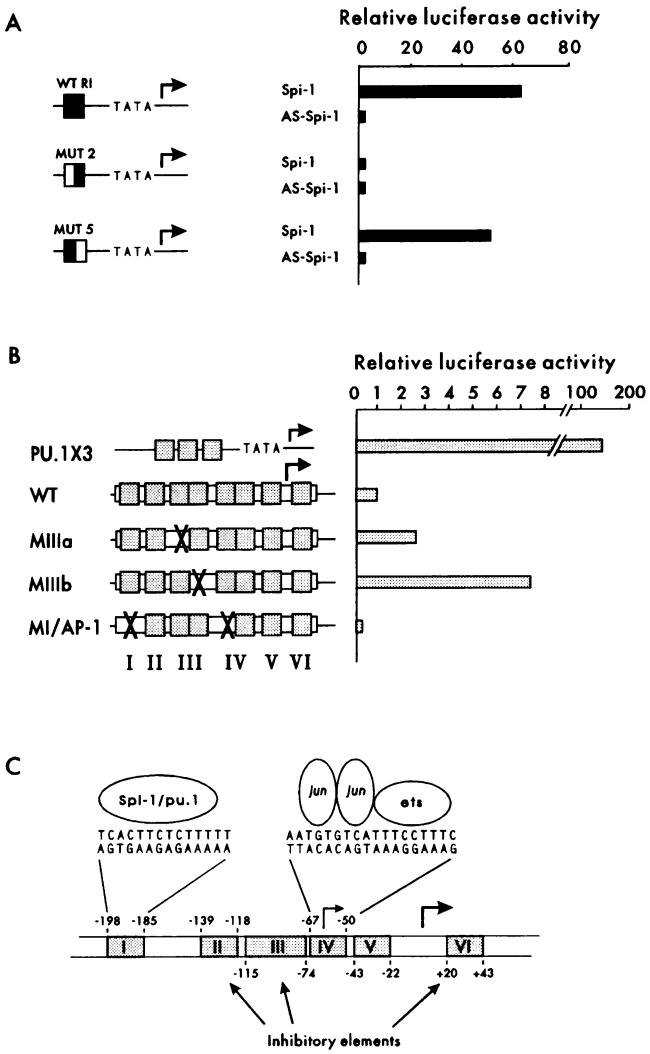

FIG. 9. Positive transcriptional control of the macrophage SR is dependent on the combinatorial interactions of multiple positive and negative factors. (A) Spi-1 activates the transcription of promoters containing the SR Spi-1/PU.1 binding site. The reporter plasmids described in Fig. 6A, consisting of wild-type region I (WT RI), mutant 2 (MUT 2), or mutant 5 (MUT 5) linked to the prolactin minimal reporter were transfected into HeLa cells with an expression vector directing the expression of either the sense or antisense (AS) strand of Spi-1. Spi-1-dependent transcriptional activation was dependent on the region I element and demonstrated the same sequence requirements observed in THP-1 cells. (B) Restriction of SR promoter activity in a B-cell line in part reflects transcriptional repression mediated through region III. The indicated promoter constructs described in Fig. 4 and a construct containing three copies of a consensus PU.1 binding site linked to the minimal prolactin promoter were transfected into S194 B cells. Luciferase activity was determined 48 h later. The results illustrated in panels A and B are each representative of three independent experiments. (C) Summary of the transcriptional control elements and *trans*-acting factors regulating the expression of the macrophage SR promoter. Regions I and IV account for the majority of positive regulation in the SR promoter. Region IV is recognized by a ternary complex consisting of c-Jun, JunB, and an ets2-like protein (40). The ets motif-binding protein appears to account for a significant fraction of the basal expression of the SR promoter and is preferentially expressed in monocyte/macrophage-like cells. The ternary complex is also a target for transcriptional activation following stimulation of THP-1 cells with activators of protein kinase C. Region I is recognized by Spi-1/PU.1 and appears to play an important role in establishing macrophage-specific expression of the SR promoter.

to those observed for the endogenous activity present in THP-1 cells (Fig. 6A).

The observation that PU.1/Spi-1 mediated transcriptional activation through the region I element raised the question of why the expression of the SR promoter was restricted from B-cell lines, such as S194 cells (Fig. 1), which express PU.1. PU.1 could be shown to be functional in these cells because a minimal promoter containing three copies of the consensus PU.1 recognition motif directed high levels of luciferase activity (Fig. 9B). This finding suggested that the low levels of expression of the SR promoter in these cells might reflect the actions of transcriptional repressors. To examine this possibility, the activities of SR promoters containing point mutations in regions I through VI were assayed. Clustered point mutations within region III that resulted in a 1.5- to 2-fold increase in SR promoter activity in THP-1 cells (Fig. 4A) resulted in 5 to 7-fold increases in SR promoter activity in S194 cells. These results are consistent with the hypothesis that B cells contain factors that function to inhibit SR gene transcription.

DISCUSSION

The SR is representative of proteins that are highly restricted in their patterns of expression to cells of monocytic origin and become markedly upregulated during the process of macrophage differentiation. In this analysis of SR gene regulation, the most proximal promoter sequences from −245 to +46 were sufficient to direct preferential expression of a reporter gene to monocyte/macrophage-derived cells. DNA binding studies and mutational analysis of the SR promoter revealed that this profile of activity reflected complex interactions between positive and negative regulatory elements (Fig. 9C). DNA binding activities that recognized regions II, III, and VI correlated with transcriptional inhibition of the SR promoter. The identities of the factors responsible for these inhibitory activities remain undefined. Factors binding to the region III element preferentially inhibited SR promoter activity in a B-cell line, suggesting that they contribute to the cell specificity of SR expression. In contrast, the factor or factors binding to the region VI element appeared to inhibit SR transcription in macrophage precursor cells. This binding activity was noted to decrease upon macrophage differentiation of THP-1 cells and was not present in P388D1 macrophages. Because mutation of the region VI element enhanced basal promoter activity but reduced the fold induction to TPA (Fig. 4), upregulation of the SR gene expression in THP-1 cells in response to TPA may involve the loss of a repressor activity bound to this site, in addition to enhancing the levels and activities of positively acting factors that act through upstream regulatory elements.

Positive transcriptional activation of the SR gene in THP-1 cells appeared to be primarily mediated through the DNA binding motifs of region I at −198 to −185 bp and region IV at −67 to −50 bp. Mutation of the region I element indicated that it played a major role in targeting SR expression to monocytic and macrophage-like cells. Expression screening resulted in the isolation of three lambda gt11 clones encoding Spi-1 fusion proteins that bound to the region I element with high affinity. Multiple lines of evidence support the conclusion that Spi-1 and PU.1 correspond to the proteins present in THP-1 and P388D1 cells, respectively, that were observed to bind the region I element in vitro and activated SR gene expression in vivo. Electrophoretic mobility shift assays demonstrated that the region I-binding proteins, accounting for complexes Ia, Ib, and Ic, bound with similar affinities to previously characterized PU.1 binding sites. In vitro-translated PU.1 bound to the region I element with high affinity and comigrated with the P388D1 Ic complex. PU.1-specific antibodies recognized the Ia, Ib, and Ic complexes, and mutational analysis of the region I binding site indicated that these complexes correlated with transcriptional activity. Finally, expression of Spi-1 in cell lines that do not contain endogenous Spi-1 resulted in transactivation of promoters containing the region I element.

The Spi-1/PU.1 transcription factor is likely to play an important role in determining the cell-specific expression of the SR gene, given its restricted pattern of expression to macrophages and B cells. The PU.1 transcription factor has been shown to be an important regulator of B-cell target genes, including immunoglobulin kappa and lambda genes (5, 24) and the immunoglobulin J-chain gene (33). The analysis of these and other genes has led to the development of a consensus recognition site of core sequence GAGGAA (37). In the case of the kappa 3′ enhancer element, and probably also for the lambda B element in the lambda 2-4 enhancer, PU.1 recruits the binding of a second B-cell-restricted factor, termed NF-EM5, that recognizes an adjacent site and requires protein-protein interactions to form a stable protein-DNA complex (24). The PU.1-NF-EM5 interaction was shown to be regulated by a phosphorylation event at serine 148 that is necessary for efficient transcriptional activation of the kappa 3′ enhancer sequence (25). In contrast, the JB element of the immunoglobulin J-chain promoter appears to bind PU.1 as a monomer (33). From cotransfection experiments with Spi-1 expression plasmids, the pattern of complexes observed with crude nuclear extracts and in vitro-translated PU.1 in electrophoretic mobility shift assays, and a detailed mutational analysis of the region I element, it is also likely that Spi-1/PU.1 binds as a monomer to the region I element in the SR promoter.

The role of Spi-1/PU.1 in the regulation of macrophage gene expression is less well defined. Recently, PU.1 was demonstrated to bind to an element from −26 to +2 in the CD11b promoter and appears to play an important role in CD11b expression (22). PU.1 core recognition motifs have also been identified in a number of other promoters active in macrophages, including the CD11a (31), CD11c (17), and macrophage inflammatory protein alpha (10). Direct evidence that PU.1 binds to these elements and is required for promoter function has not been clearly established, however. The present studies demonstrate that the macrophage SR gene represents an important target of PU.1 and support a general role of PU.1 in directing the expression of a network of genes that are required for monocyte/macrophage function.

The PU.1 binding site within the SR promoter is divergent from the GGAA consensus that has generally been regarded to form the core of the PU.1 recognition motif. Intriguingly, the CD11b and JB binding sites exhibit the same divergent nucleotide within the core (Fig. 7C) (22, 33). The binding affinity of PU.1 for the SR region I sequence was noted to be similar to that of the PU.1 element present in the immunoglobulin kappa 3′ enhancer and an idealized PU.1 binding site, indicating that the divergent core recognition sequence is compatible with high-affinity binding. Alignments of the SR region I element with the JB and CDIIb PU.1 sites containing the AGAA core demonstrate a highly conserved sequence 5′ of the divergent core motif (Fig. 7C). The upstream purine rich sequences are necessary for high-affinity binding in the context of the altered core motif, as illustrated by the complete loss of binding when these bases are mutated (Fig. 5). These purine residues have been identified as protein-DNA contact points in dimethyl sulfate interference studies (33). In addition, the G residue at

position 11 downstream from the AGAA core appears to be an additional important determinant of PU.1 binding.

Intriguingly, the region I element was found to be a relatively low affinity binding site for Spi-B, and PU.1/Spi-1-specific antibodies excluded it as a significant binding activity in nuclear extracts derived from P388D1 macrophages and THP-1 cells. In contrast, Spi-B was found to bind with relatively high affinity to a consensus PU.1/Spi-1 recognition element. The region I element of the SR promoter therefore represents a functionally important subclass of PU.1 binding sites in that it permits a relative level of discrimination between the binding of PU.1/Spi-1 and Spi-B. These observations support the idea that Spi-B and PU.1/Spi-1 regulate overlapping but nonidentical sets of genes and have different roles in hematopoiesis (26).

Positive transcriptional control of the SR promoter was also dependent on a composite binding site for AP-1 and ets domain transcription factors. Selective mutation of the ets motif resulted in a substantial decrease in basal SR promoter function, while a mutation of the AP-1 site affected basal promoter activity only in the context of a second mutation in the region I sequence (Fig. 4A). In contrast, both the AP-1 and ets motifs were found to be critical for the transcriptional response of the SR gene to TPA (40) (Fig. 4B). Although not exclusively restricted to macrophages, nuclear extracts containing proteins capable of forming ternary complexes on the region IV element were derived from a relatively limited number of cell types. These observations suggest that cooperativity between AP-1 and ets domain proteins further contributes to the cell specificity of SR expression.

Scavenger receptor expression has been reported to be stimulated in primary monocytes by M-CSF and granulocyte/macrophage colony-stimulating factor (GM-CSF) (13). Furthermore, we have recently demonstrated that M-CSF and GM-CSF dramatically stimulate SR expression in mouse bone marrow progenitor cells (11a). These observations suggest a link between the GM-CSF and M-CSF receptors and transcriptional activation of the SR gene. Treatment of monocytes with M-CSF directly activates Ras-dependent signal transduction pathways and has been noted to increase protein kinase C activity (2, 12). Both of these signal transduction systems are known to regulate transcriptional via AP-1/ets motifs (28, 38). The AP-1/ets motif of region IV, in concert with additional AP-1/ets motifs located 4 kb upstream, are therefore likely to mediate the transcriptional response of the SR to M-CSF. Consistent with this possibility, we have recently demonstrated that Ras, c-Jun and ets2 synergistically activate transcription from the region IV element (40).

It is intriguing that the M-CSF receptor is likely to be a PU.1 target gene, given the presence of several conserved PU.1 binding sites within the M-CSF receptor promoter (29) and the ability of PU.1 to transactivate the PU.1 promoter in nonmacrophage cells (41). PU.1 may therefore act as both a direct and an indirect regulator of macrophage gene expression. By positively regulating the expression of the M-CSF receptor, PU.1 would function to promote the responsiveness of monocytes to M-CSF. In the case of the SR gene, this sequence of events would be hypothesized to establish progressively increased levels of SR expression during macrophage differentiation. Initially, PU.1 could directly activate basal levels of expression in monocytes and subsequently facilitate a second level of transcriptional control that is contingent on the external milieu (i.e., M-CSF dependent). This sort of hierarchical control may provide the basis for context-dependent control of macrophage gene expression.

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REFERENCES

1. **Auwerx, J. H., S. Deeb, J. D. Brunzell, R. Peng, and A. Chait.** 1992. Transcriptional activation of the lipoprotein lipase and apolipoprotein E genes accompanies differentiation in some human macrophage-like cell lines. Biochemistry **27**:2651–2655.
2. **Bortner, D. M., M. Ulivi, M. F. Roussel, and M. C. Ostromski.** 1991. The carboxy-termal catalytic domain of the GTPase-activating protein inhibits nuclear signal transduction and morphological transformation mediated by the CSF-1 receptor. Genes Dev. **5**:1777–1785.
3. **Chen, C., and H. Okayama.** 1987. High-efficiency transformation of mammalian cells by plasmid DNA. Mol. Cell. Biol. **7**:2745–2752.
4. **deWet, J. R., K. V. Wood, M. DeLuca, D. R. Helinski, and S. Subramani.** 1987. Firefly luciferase gene: structure and expression in mammalian cells. Mol. Cell. Biol. **7**:725–737.
5. **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 γ2-4 enhancer. Mol. Cell. Biol. **13**:6452–6461.
6. **Fogelman, A. M., M. E. Haberland, J. Seager, M. Hokom, and P. A. Edwards.** 1981. Factors regulating the activities of the low density lipoprotein receptor and the scavenger receptor on human monocyte-macrophages. J. Lipid Res. **22**:1131–1141.
7. **Freeman, M., Y. Ekkel, L. Rohrer, M. Penman, N. J. Freedman, G. M. Chisolm, and M. Krieger.** 1991. Expression of type I and type II bovine scavenger receptors in Chinese hamster ovary cells: lipid droplet accumulation and nonreciprocal cross competition by acetylated and oxidized low density lipoprotein. Proc. Natl. Acad. Sci. USA **88**:4931–4935.
8. **Fried, M., and D. M. Crothers.** 1987. Equilibria and kinetics of lac repressor-operator interactions by PAGE. Nucleic Acids Res. **9**:6505–6525.
9. **Goldstein, J. L., Y. K. Ho, S. K. Basu, and M. S. Brown.** 1979. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. Proc. Natl. Acad. Sci. USA **76**:333–337.
10. **Grove, M., and M. Plumb.** 1993. C/EBP, NF-κB, and c-Ets family members and transcriptional regulation of the cell-specific and inducible macrophage inflammatory protein 1-alpha immediate-early gene. Mol. Cell. Biol. **13**:5276–5289.
11. **Hara, H., H. Tanishita, S. Yokoyama, S. Tajima, and A. Yamamoto.** 1987. Induction of acetylated low density lipoprotein receptor and suppression of low density lipoprotein receptor on the cells of human monocytic leukemia cell line (THP-1 cell). Biochem. Biophys. Res. Commun. **146**:802–808.

11a. **Horvai, A., and C. K. Glass.** Unpublished data.

12. **Imamura, K., A. Dianoux, T. Nakamura, and D. Kufe.** 1990. Colony-stimulating factor-1 activates protein kinase C in human monocytes. EMBO J. **9**:2423–2428.
13. **Ishibashi, S., T. Inaba, H. Shimano, K. Harada, I. Inoue, H. Mokuno, N. Mori, T. Gotoda, F. Takaku, and N. Yamada.** 1990. Monocyte colony-stimulating factor enhances uptake and degradation of acetylated low density lipoproteins and cholesterol esterification in human monocyte-derived macrophages. J. Biol. Chem. **265**:14109.
14. **Klemsz, M. J., S. R. McKercher, A. Celada, C. V. 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.
15. **Kodama, T., M. Freeman, L. Rohrer, J. Zabrecky, P. Matsudaira, and M. Krieger.** 1990. Type I macrophage scavenger receptor contains alpha-helical and collagen-like coiled coils. Nature (London) **343:**531–535.
16. **Krieger, M.** 1992. Molecular flypaper and atherosclerosis: structure of the macrophage scavenger receptor. Trends Biochem. Sci. **17:**141–146.
17. **Lopez-Cabrera, M., A. Nueda, A. Vara, J. Garcia-Aguilar, A. Tugores, and A. L. Corbi.** 1993. Characterization of the p150,95 leukocyte integrin alpha subunit (CD11c) gene promoter. J. Bio. Chem. **268:**1187–1193.
18. **Malassine, A., E. Alsat, C. Besse, R. Rebourcet, and L. Cedard.** 1990. Acetylated low density lipoprotein endocytosis by human syncytiotrophoblast in culture. Placenta **11:**191–204.
19. **Moulton, K. S., H. Wu, J. Barnett, S. Partharathy, and C. K. Glass.** 1992. Regulated expression of the acetylated LDL receptor gene and isolation of promoter sequences. Proc. Natl. Acad. Sci. USA **89:**8102–8106.
20. **Nagelkerke, J. F., K. P. Barto, and T. J. C. Van Berkel.** 1983. *In vivo* and *in vitro* uptake and degradation of acetylated low density lipoprotein by rat liver endothelial, Kupffer, and parenchymal cells. J. Biol. Chem. **258:**12221–12227.
21. **Naito, M., T. Kodama, A. Matsumoto, T. Doi, and K. Takahashi.** 1991. Tissue distribution, intracellular localization, and in vitro expression of bovine macrophage scavenger receptors. Am. J. Pathol. **139:**1411–1423.
22. **Pajl, H. L., R. L. Scheibe, D. Zhang, H. 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.
23. **Pitas, R. E., J. Boyles, R. W. Mahley, and D. M. Bissell.** 1985. Uptake of chemically modified low density lipoproteins in vivo is mediated by specific endothelial cells. J. Cell Biol. **100:**103–117.
24. **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 K 3′ enhancer activity. Mol. Cell. Biol. **12:**368–378.
25. **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 **259:**1622–1625.
26. **Ray, D., R. Bosselut, J. Ghysdael, M. G. Mattei, A. Tavitian, and F. M. Gachelin.** 1992. Characterization of Spi-B, a transcription factor related to the putative oncoprotein Spi-1/PU.1. Mol. Cell. Biol. **12:**4297–4304.
27. **Ray, D., S. Culine, A. Tavitian, and F. M. Gachelin.** 1990. The human homologue of the putative proto-oncogene Spi-1: characterization and expression in tumors. Oncogene **5:**663–668.
28. **Reddy, M. A., S. J. Langer, M. S. Colman, and M. C. Ostrowski.** 1992. An enhancer element responsive to *ras* and *fms* signaling pathways is composed of two distinct nuclear factor binding sites. Mol. Endocrinol. **6:**1051–1060.
29. **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.
30. **Rohrer, L., M. Freeman, T. Kodama, M. Penman, and M. Krieger.** 1990. Coiled-coil fibrous domains mediate ligand binding by macrophage scavenger receptor type II. Nature (London) **343:** 570–572.
31. **Shelley, C. S., O. C. Farokhzad, and M. A. Arnaout.** 1993. Identification of cell-specific and developmentally regulated nuclear factors that direct myeloid and lymphoid expression of the CD11a gene. Proc. Natl. Acad. Sci. USA **90:**5364–5368.
32. **Sherr, C. J.** 1990. Regulation of mononuclear phagocyte proliferation by colony-stimulating factor 1. Int. J. Cell Cloning **8:**46–62.
33. **Shin, M. K., and M. E. Koshland.** 1993. Ets-related protein PU.1 regulates expression of the immunoglobulin J-chain gene through a novel Ets-binding element. Genes Dev. **7:**2006–2015.
34. **Singh, H., R. G. Clerc, and J. H. LeBowitz.** 1989. Molecular cloning of sequence-specific DNA binding proteins using recognition site probes. BioTechniques **7(3):**252–261.
35. **Steinberg, D., S. Parthasarathy, T. E. Carew, J. C. Khoo, and J. L. Witztum.** 1989. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N. Engl. J. Med. **320:**915–924.
36. **Vinson, C. R., K. L. LaMarco, P. F. Johnson, W. H. Landschulz, and S. L. McKnight.** 1988. In situ detection of sequence-specific DNA binding activity specified by a recombinant bacteriophage. Genes Dev. **2:**801–806.
37. **Wasylyk, B., S. L. Hahn, and A. Giovane.** 1993. The Ets family of transcription factors. Eur. J. Biochem. **211:**7–18.
38. **Wasylyk, B., C. Wasylyk, P. Flores, A. Begue, D. Leprince, and D. Stehelin.** 1990. The c-*ets* proto-oncogenes encode transcription factors that cooperate with c-Fos and c-Jun for transcriptional activation. Nature (London) **346:**191–193.
39. **Wood, K. V.** 1991. Bioluminescence and chemiluminescence: current status. John Wiley & Sons, Chichester, England.
40. **Wu, H., K. Moulton, A. Horvai, S. Parik, and C. K. Glass.** 1994. Combinatorial interactions between AP-1 and ets domain proteins contribute to the developmental regulation of the macrophage scavenger gene. Mol. Cell. Biol. **14:**2129–2139.
41. **Zhang, D., C. J. Hetherington, H. 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.