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Identification of cell-specific and developmentally regulated nuclear factors that direct myeloid and lymphoid expression of the CD11a gene

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ABSTRACT **Human CD11a/CD18 is a noncovalently associated heterodimeric receptor expressed exclusively on the surface of lymphocytes and myeloid cells. To begin to understand the mechanisms that direct the expression of the genes encoding this receptor, we have cloned and characterized the promoter region of the CD11a gene and localized cis-acting elements involved in its expression in lymphoid and myeloid cells. One such element is the "LYM" box, which interacts with two sets of DNA-binding activities, one primarily expressed in lymphocytes and preerythroid cells and the other expressed predominantly in myeloid cells. A second element required for expression of the CD11a gene contains the "GAGA" sequence RRRGAGGAAG (R indicates a purine), which interacts with the DNA-binding activities MS-1 and MS-2. MS-1 is expressed exclusively in myeloid cells and probably represents a member of the Ets family of transcription activators. MS-2 is present in epithelial, preerythroid, and lymphoid cells but is only detected in myeloid cells after differentiation. MS-2 also binds to a second element within the CD11a promoter and homologous elements present in the promoter regions of the CD11b and CD43 genes. Since MS-2 interacts with a number of different gene promoters and is developmentally regulated in myeloid cells, it may play a major role in regulating myeloid gene expression.**

The β_2 integrins mediate a number of vital leukocyte functions and consist of three heterodimers composed of distinct α subunits (CD11a, CD11b, or CD11c) associated with a common β subunit (CD18) (1–4). The genes encoding these cell adhesion molecules exhibit distinct but overlapping patterns of cell- and development-specific expression (1). Although virtually all leukocytes express CD11a and CD18, expression of the CD11b and CD11c genes is largely restricted to myelomonocytic cells. The CD11 genes have been mapped to the p11–p13.1 region of chromosome 16 (5), which has been implicated in chromosomal abnormalities in patients with acute myelomonocytic leukemia (6, 7). Elucidation of the molecular mechanisms underlying the regulation of the CD11 and CD18 genes in leukocytes requires the identification and analysis of the cis- and trans-acting elements involved. In this report we describe the characterization of the CD11a gene promoter.† Cis-acting elements involved in lymphoid and myeloid expression have been identified and putative transcription factors with which they interact have been characterized.

MATERIALS AND METHODS

Isolation of the 5′ End of the CD11a Gene. Oligonucleotide AA1 (5′-TCAGGAGGCCCGTGGGATTT-3′) designed from the 5′ end of a human CD11a cDNA clone (8) was used to screen (9) a human genomic library [American Type Culture Collection (ATCC) number 57758]. One clone, λCD11a, was isolated and from it an 8-kb *Bgl* II fragment was ligated into the *Bam*HI site of pGEM-7Zf+ (Promega) to generate the subclone pZ11a-8 (Fig. 1).

DNA Sequencing. Double-stranded DNA was sequenced by the dideoxynucleotide chain-termination/extension method (10). A series of specific oligonucleotide primers based on the CD11a cDNA sequence (8) and the CD11a gene sequence, as it became known, were used to sequence the regions of pZ11a-8 indicated in Fig. 1.

Primer-Extension Analysis. The site of transcription initiation was determined by primer extension using the oligonucleotide AA2 (5′-AGGGGCAGCGTCAGGAGG-3′). AA2 was 5′-end-labeled using T4 polynucleotide kinase and [γ-^{32}P]ATP and extended (11) using as template 20 μg of total RNA isolated from human monocytes.

Cell Culture. Cell lines were obtained from ATCC and grown according to their specifications. Phorbol 12-myristate 13-acetate was obtained from Sigma and used at 100 ng/ml to induce the differentiation of U937 cells.

Plasmid Construction. The activity of the CD11a promoter was assessed using the expression vector pATLuc that contains a promoterless firefly luciferase reporter gene. This vector was constructed by replacing the 1.4-kb *Pst* I–*Cla* I fragment of pA$_3$LUC (12) with the 1.4-kb *Pst* I–*Cla* I fragment of poLUC (13). The PCR and restriction endonuclease digestion were used to generate three fragments of the CD11a gene from pZ11a-8, representing nt −525 to +103, nt −140 to +103, and nt −116 to +103 relative to the transcription initiation site. These fragments were then subcloned into the filled-in *Hin*dIII site of pATLuc to generate, respectively, pLaI–III (see Fig. 5). The construct pLaIV was produced by subcloning PCR-generated CD11a gene fragments representing nt −525 to −85 and nt −74 to +103 into, respectively, the filled-in *Sal* I and *Hin*dIII sites of pATLuc (see Fig. 5).

Transfection. Cells were transfected (13, 14) with a luciferase test plasmid plus the plasmid pCH110 (Pharmacia LKB Biotechnology) containing the *lacZ* gene. Each transfection of pLaI–IV was performed in parallel with a transfection of the negative control plasmid pATLuc. Sixteen hours after electroporation, cells were processed for assay of β-galactosidase and luciferase activity (13).

Electrophoretic-mobility-shift assays (EMSAs). Nuclear extracts were prepared essentially as described by Dignam *et al.* (15). DNA probes were generated by annealing complemen-

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Abbreviations: NF-1, nuclear factor 1; EMSA, electrophoretic-mobility-shift assay.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M95609).

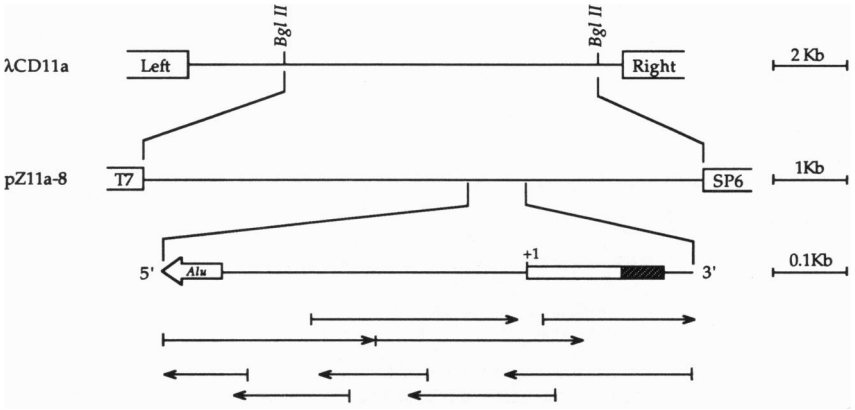

FIG. 1. Partial restriction map of λCD11a showing only those *Bgl* II sites used in generating the subclone pZ11a-8. The area of pZ11a-8 that was sequenced is expanded to show the position and orientation of the *Alu* repeat in the 5′ flanking region relative to the first exon. The open and shaded boxes represent, respectively, the noncoding and coding regions of the first exon that begins at the transcription initiation site marked +1. The strategy used in sequencing is depicted by arrows denoting the length and direction of individual sequencing reaction products.

tary oligonucleotides that had been radiolabeled at their 5′ ends using T4 polynucleotide kinase and [γ-^{32}P]ATP. Nuclear extract (1–3 μg) was incubated at 4°C for 15 min in 70 mM KCl/5 mM NaCl/20 mM Tris Cl, pH 7.5/0.5 mM EDTA/1 mM dithiothreitol/10% (vol/vol) glycerol/2.4 μg of poly[d(I-C)]·poly[d(I-C)]/a 250–500 molar excess of unlabeled competitor probe where indicated. Radiolabeled probe was then added and the incubation was continued for 30 min prior to gel electrophoresis and autoradiography.

RESULTS AND DISCUSSION

Isolation of the 5′ End of the CD11a Gene. A human genomic library was screened with the oligonucleotide AA1 and one recombinant, λCD11a, was isolated. An 8-kb *Bgl* II fragment of λCD11a was subcloned into pGEM-7Zf+ to generate pZ11a-8 (Fig. 1) and the region containing the 5′ end of the CD11a gene was sequenced (Fig. 2).

Transcription Initiation Site and First Exon. Primer-extension analysis (Fig. 3) established that transcription of the CD11a gene is initiated at a single major site, the thymidine designated nt 1 in Fig. 2. Comparison of the CD11a genomic and cDNA sequences indicates that the first exon of the CD11a gene consists of 194 bp including 133 bp of 5′ noncoding sequence and 61 bp encoding the first 20 aa of the CD11a leader polypeptide. The 5′ end of the first intron of the CD11a gene is in general agreement with the consensus sequence derived from the equivalent position in other eukaryotic genes (16).

5′ Flanking Region. Upstream from the transcription initiation site, 525 nt have been sequenced (Fig. 2). Between nt −525 and −442 there is the 5′ end of an *Alu* repeat that is inverted with respect to the CD11a gene. In the presumed CD11a promoter region, there are no "TATA" or "CAAT" boxes; however, between nt −40 and −31, where a TATA box would be expected, there is an overlapping duplication of the sequence CCTCCC, which has been shown to be important to the promoter activity of other genes (17, 18). In the antisense strand beginning at nt −75, there is the sequence AGGGAGGAAG, which conforms to what we refer to as the "GAGA" motif that is often found associated with myeloid expressed genes (Fig. 4). This contains not only a CCTCCC element but also the sequence GGAA, a potential binding site for the Ets family of transcription factors (29), and the sequence GAGGAA, a potential binding site for the Ets family member PU-1 (30). Other sequences of note in the 5′ flanking region include GCAGGCACA directly repeated at nt −117 and −49, which contains a region homologous to the recognition site of the nuclear factor 1 (NF-1) proteins (31, 32). An exact match to the NF-1 binding site is present in the antisense strand between nt −26 and −22. In addition, between nt −135 and −129, there are the overlapping sequences GGATGA and TGATGT homologous to the consensus target sites of both the "GATA" and Ets families of transcription factors (29, 33–35). Expression of CD11a on the surface of T cells increases during mitogen or antigen stimulation (36). In this regard it is interesting to note in the antisense strand a direct repeat of the sequence CATT(A/T) starting at nt −121 and −102, which has been shown to be required for mitogen-inducible gene expression (37). During the granulocytic differentiation of HL-60 cells induced by retinoic acid, surface expression of CD11a decreases (38). Three T(G/C)AC(C/A) motifs representing possible retinoic acid response elements are present in the sense strand beginning at nt −18, −155, and −182 and three additional

FIG. 2. Nucleotide sequence of the 5′ end of the human CD11a gene. The first exon begins at the transcription initiation site designated +1 and ends at nt +194 after which the first intron is depicted in lowercase type. Above the coding portion of the first exon, the derived CD11a amino acid sequence is indicated in single-letter code. The *Alu* sequence is underlined. The LYM box is indicated by a boldface line above the sequence. Boxed are the sites that interact with the DNA-binding activities MS-1 and MS-2. The conserved GAGA motif containing binding sites for both MS-1 and MS-2 is underlined with a striped box. In the sense strand, the repeats containing homology to the recognition site of NF-1 are indicated by solid arrows pointing to the right below the sequence. In the antisense strand, an exact match to the NF-1 binding site is indicated by a solid arrow pointing to the left below the sequence. Open arrows below the sequence indicate a direct repeat that may mediate the induction of the CD11a gene by mitogen (see text).

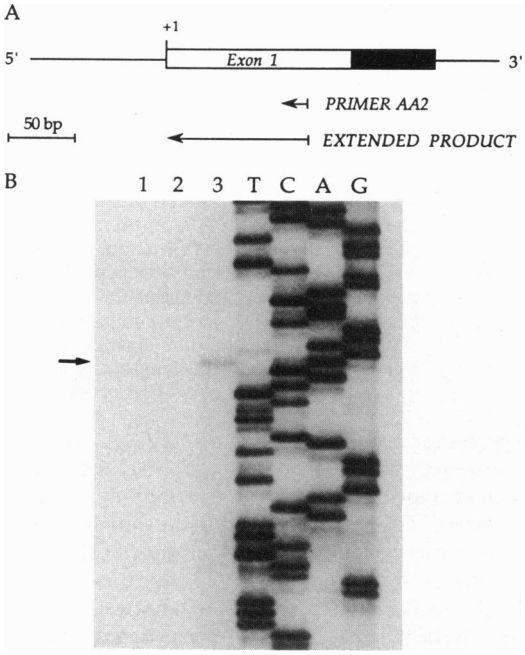

FIG. 3. Identification of the transcription initiation site of the CD11a gene by primer-extension analysis. (*A*) The 5′ end of the gene is represented at the top; the open and shaded boxes indicate, respectively, the noncoding and coding regions of the first exon and lines represent 5′ flanking and intron sequences. The major transcription initiation site identified by primer-extension analysis is marked +1. The oligonucleotide primer AA2 used in the primer-extension analysis is represented below as is the product of its extension with reverse transcriptase. This primer was also used to generate reference dideoxynucleotide sequencing products of the CD11a gene. (*B*) Lanes: 1, oligonucleotide AA2 used as primer that was not extended; 2, product of primer extension using 10 μg of yeast tRNA; 3, product of extension using 10 μg of total human monocyte RNA; T, C, A, and G, reference dideoxynucleotide sequencing reaction products of the noncoding strand of the CD11a gene generated by using AA2 as the primer. The arrow marks the 103-nt fragment generated by primer extension that in the coding strand aligns with the thymidine residue at nt +1 (Fig. 2).

motifs are present in the antisense strand beginning at nt −16, −126, and −143.

Identification of Cis-Acting Elements Involved in Myeloid and Lymphoid Expression. To analyze the 5′ flanking region of the CD11a gene for promoter activity, four constructs were generated, pLaI–IV (Fig. 5). Transfection of pLaI into CEM T lymphocytes or U937 monoblastic cells resulted in luciferase activities that were on average 36- and 104-fold above background, respectively. The pLaII construct resulted in similar activities thus limiting the promoter region to within 140 nt upstream of the transcription initiation site. Deletion of the 24-nt LYM box (Fig. 2) severely reduced the level of expression in CEM cells but only slightly affected expression in U937 cells (Fig. 5). The LYM box, therefore, contributes mainly to the lymphocytic activity of the CD11a gene. This element contains the overlapping sequences GGATGA and TGATGT, which resemble the consensus binding sites of the GATA and Ets families of transcription activators. The LYM box also contains the most 5′ of the two CATT(A/T) sequences, which may mediate the induction of the CD11a gene by mitogen. Comparison of the CD11a gene with others expressed in myeloid cells revealed the conserved sequence RRRGAGGAAG (Fig. 4). In pLaIV this GAGA element (nt −84 to −75) is specifically deleted from pLaI, resulting in reduced expression in CEM and U937 cells (Fig. 5). The myeloid and lymphoid expression of the CD11a gene, therefore, involves at least two cis-acting elements. The GAGA element appears to be critical for expression in both lymphoid and myeloid cells and the LYM box appears vital only to lymphoid expression.

CD11a	−70	G G T T C	A G G G A G G A A G	T G T A A	−89
CD11b	−133	A A G C T	G G G G A G G A A G	G G T G G	−114
CD18	−672	A G G A G	G A G G A G G A A G	A G G A G	−691
α4	−64	A G T G T	G G G G A G G A A G	G A A G T	−45
FcγRI	+31	T T A G A	A A A G A G G A A G	G A A A T	+12
Cathepsin G	−32	C C C T G	A G A G A G G A A G	G A A G G	−51
Cathepsin G	−23	T C T A G	G A G G A G G A A G	C A C A G	−4
Neutrophil Elastase	−98	C C A G G	G G A G A G G A A G	T G G A G	−79
c-fes/fps	−81	C G C G G	G A G G A G G A A G	C G C G G	−62
CD13	−599	C C C A G	G A G G A G G A A G	T T G C A	−580
MRP8	−174	A G C G G	A A A G A G G A A G	G C A T T	−193
IL-1β	−1911	A A A A G	A A A G A G G A A G	G A A G G	−1892
Type IV Collagenase	−549	A G C A G	G G A G A G G A A G	C T G A G	−530
"GAGA" Consensus			R R R G A G G A A G		

FIG. 4. Comparison of the GAGA elements (boxed) associated with human genes expressed in myeloid cells. The numbers indicate the position of each element relative to the major site of transcription initiation (13, 19–28). The GAGA element of the gene for the human Fc receptor for IgG FcγRI is also found in the genes for murine FcγRI and FcγRIII (24). R indicates a purine. MRP8, macrophage migration inhibitory factor-related protein 8; IL-1β, interleukin 1β.

Characterization of Trans-Acting Factors. To characterize the putative transcription factors that interact with the LYM box and the GAGA elements (Fig. 2), EMSAs were performed. Multiple protein complexes form over the LYM box region. In U937 cells, two complexes, GS-3 and GS-4, predominate and in B lymphocytes and preerythroid cells two others, GS-1 and GS-2, are the major complexes observed

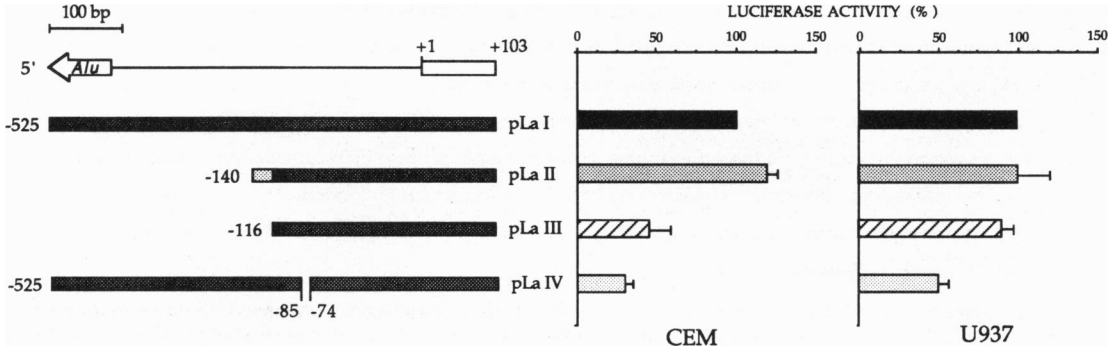

FIG. 5. (*Left*) Deletion analysis of the CD11a promoter. The portions of the CD11a gene used in transfection assays are illustrated. The lightly shaded area of pLaII represents the LYM box (Fig. 2). The GAGA element (nt −84 to −75) is specifically deleted in pLaIV. (*Right*) Luciferase reporter gene activity, corrected for transfection efficiency, is expressed after subtraction of the background activity conferred by the negative control plasmid pATLuc. The level of expression of pLaI is assigned an arbitrary value of 100% and the expression level conferred by the deletion constructs is displayed as a proportion of this value. Each histogram represents the mean ± SD of two transfection experiments.

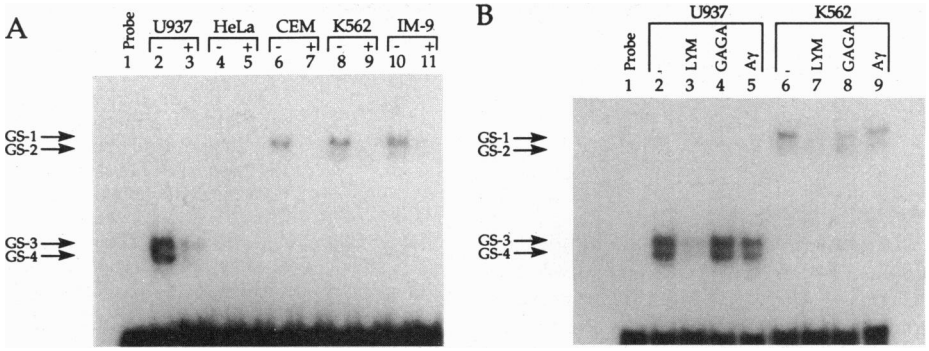

FIG. 6. EMSA of the nuclear proteins that interact with the LYM box. (*A*) A radiolabeled DNA fragment, D/D3′, representing the LYM box (Fig. 2) was incubated with no nuclear extract (lane 1) or nuclear extracts prepared from U937 monoblastic cells (lanes 2 and 3), HeLa epithelial cells (lanes 4 and 5), CEM T-lymphoblastoid cells (lanes 6 and 7), K562 preerythroid cells (lanes 8 and 9), or IM-9 B lymphoblastoid cells (lanes 10 and 11). Binding reactions were performed either in the absence (−) of unlabeled specific competitor DNA (lanes 2, 4, 6, 8, and 10) or in the presence (+) of a 500 molar excess of unlabeled D/D3′ (lanes 3, 5, 7, 9, and 11). The positions of the protein–DNA complexes, GS-1, GS-2, GS-3, and GS-4, are marked with arrows. (*B*) Radiolabeled D/D3′ incubated with no nuclear extract (lane 1) or nuclear extracts prepared from U937 cells (lanes 2–5) or K562 cells (lanes 6–9) in the absence of unlabeled specific competitor DNA (lanes 2 and 6) or a 500 molar excess of the following unlabeled competitor DNA fragments. Lanes: 3 and 7, D/D3′; 4 and 8, A/A3′, spanning nt −89 to −70 of the CD11a promoter and containing the GAGA element; 5 and 9, the Aγ-globin promoter fragment 5′-ACACTATCTCAATGCAAATATCTGT-3′ that binds the GATA family of transcription factors (39).

(Fig. 6*A*). T lymphocytes express primarily GS-1 and the epithelial cell line HeLa expresses no detectable nuclear protein that interacts with the LYM box. Despite containing sequences homologous to the recognition site of the GATA family of transcription factors, competition assays (Fig. 6*B*, lanes 5 and 9) demonstrate that none of the four protein complexes that interact with the LYM box represents a known member of this group of molecules. The GAGA

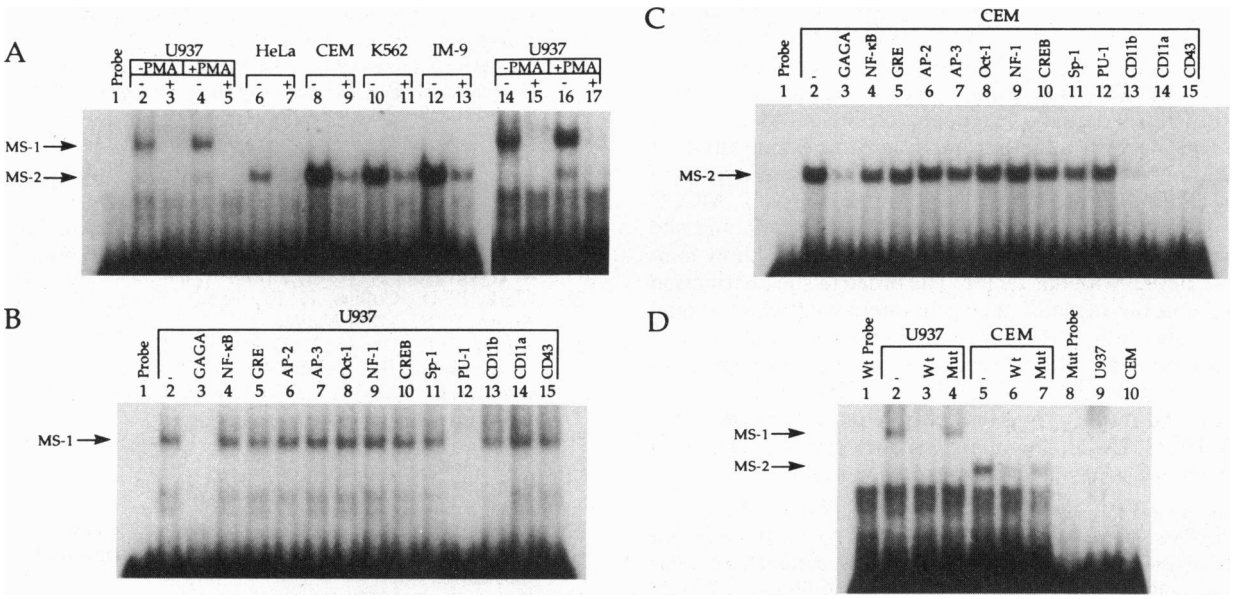

FIG. 7. EMSA of the nuclear proteins that interact with the GAGA element. (*A*) A radiolabeled DNA fragment, A/A3′, containing the conserved GAGA motif was incubated with no nuclear extract (lane 1) or nuclear extracts prepared from uninduced (−PMA) U937 cells (lanes 2 and 3) or phorbol 12-myristate 13-acetate-induced (+PMA) U937 cells (lanes 4 and 5), HeLa (lanes 6 and 7), CEM (lanes 8 and 9), K562 (lanes 10 and 11), or IM-9 (lanes 12 and 13) cells. Binding reactions were performed either in the absence (−) of unlabeled specific competitor DNA (lanes 2, 4, 6, 8, 10, and 12) or the presence (+) of a 250 molar excess of unlabeled A/A3′ (lanes 3, 5, 7, 9, 11, and 13). Lanes 14–17 represent lanes 2–5 subjected to autoradiography for 24 instead of 12 h. The positions of the protein–DNA complexes, MS-1 and MS-2, are marked with arrows. The relevant portion of the gel is shown. (*B*) Radiolabeled A/A3′ incubated with no nuclear extract (lane 1) or nuclear extracts prepared from U937 cells in the absence of unlabeled specific competitor DNA (lane 2) or a 250 molar excess of the following unlabeled competitor DNA fragments. Lanes: 3, A/A3′; 4–11, DNA fragments representing the recognition sites of the indicated transcription factors; 12, the simian virus 40 DNA fragment shown to bind the murine transcription factor PU-1 (30); 13–15, homologous DNA fragments spanning, respectively, nt −132 to −115 of the CD11b promoter (13), nt −45 to −25 of the CD11a promoter, and nt −2 to +20 of the CD43 promoter (11) (Fig. 8). The position of the protein–DNA complex MS-1 is marked with an arrow. (*C*) The equivalent experiment as represented in *B* except performed with nuclear extracts prepared from CEM cells. The position of the protein–DNA complex MS-2 is marked with an arrow. (*D*) Radiolabeled A/A3′, either wild-type (Wt) or mutant [Mut, where the dinucleotide CC, common to the putative recognition sites of MS-1 and MS-2 (Fig. 2), is replaced by TT], incubated with no nuclear extract (lanes 1 and 8, respectively) or nuclear extracts prepared from U937 or CEM cells in the absence (−) or presence of a 250 molar excess of unlabeled Wt (lanes 3 and 6) or Mut (lanes 4 and 7) competitor DNA. With Wt A/A3′, protein–DNA complexes representing MS-1 and MS-2 are seen, respectively, in nuclear extracts from U937 (lane 2) and CEM (lane 5). In contrast, no similar complexes are seen when radiolabeled Mut A/A3′ is used (lanes 9 and 10). In competition studies, unlabeled Mut A/A3′ produced some inhibition of radiolabeled Wt A/A3′ binding (lanes 4 and 7), suggesting that its binding to MS-1 and MS-2 was reduced but not completely eliminated.

CD11a	-89	T T A C A C T T C C T C C C T G A A C C	-70
CD11a	-45	G C A C A C C T C C C T C C C C G C C T G	-25
CD11a	-45	G C A C A C C T C C C T C C C C G C C T G	-25
CD43	+20	C C C T G G C T C C A C C C A C C C C A C C	-2
CD43	+20	C C C T G G C T C C A C C C A C C C C A C C	-2
CD11b	-115	C A C C C T T C C T C C C C A G C T	-132
MS-2 Consensus		C C A/T C C C	

FIG. 8. Sequence comparison of the oligonucleotides that interact with MS-2. The numbers indicate the position of each oligonucleotide relative to the major site of transcription initiation (11, 13). The consensus sequence is boxed.

element of the CD11a gene interacts with a number of DNA-binding activities, two of which, MS-1 and MS-2, are characterized in this report (Fig. 7). MS-1 is expressed exclusively in myelomonocytic cells and also binds to the recognition site of the murine Ets transcription factor PU-1 (30) (Fig. 7*B*, lane 12), suggesting that MS-1 and PU-1 are related. All the members of the Ets family bind to DNA sequences that contain the core motif GGA(A/T) that is essential for DNA–protein interaction. Mutation analysis demonstrates that the two guanine residues of this motif are also critical for MS-1 binding (Fig. 7*D*, lanes 4 and 9). MS-2 is expressed in epithelial cells, B and T lymphocytes, and preerythroid cells. It is also under developmental control in myeloid cells, not being expressed until these cells have been induced to differentiate along the monocytic pathway. In U937 cells, such differentiation appears to be induced by electroporation and by phorbol ester (5). Consequently, in electroporated U937 cells, deletion of the GAGA element from the CD11a gene probably results in a loss of both MS-1 and MS-2 binding. Competition studies demonstrate that in addition to this GAGA element, MS-2 also binds to the duplicated CCTCCC element of the CD11a gene and to homologous elements within the CD11b and CD43 genes (Fig. 7*C*, lanes 13–15). Comparison of the sequences of these elements (Fig. 8) defines the recognition site of MS-2 as being CC(T/A)CCC with mutation analysis establishing the first two cytidine residues of the site as being important for protein binding (Fig. 7*D*, lanes 7 and 10). Although the CC(T/A)CCC motif shares sequence similarity with the Sp1 binding site CCGCCC, competition studies (Fig. 7*C*, lane 11) show minimal interaction with this factor. The broad tissue distribution of MS-2 and the range of gene promoters with which it may interact indicate that this represents a general transcription factor. The observations that it is under developmental control in myeloid cells and that its recognition site is almost always found in the promoters of myeloid-expressed genes (11, 13, 19–23, 25–28, 40–42) may signify that MS-2 plays an important role in myeloid gene expression.

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