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# The promoter of the CD11b gene directs myeloid-specific and developmentally regulated expression

($\beta_2$ integrins/gene structure/gene expression/transcription factors/myeloid differentiation)

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**ABSTRACT** **Human CD11b/CD18 (complement receptor type 3) is a member of the $\beta_2$ integrin subfamily which also includes the heterodimers CD11a/CD18 and CD11c/CD18. The CD11 molecules and the common CD18 are the products of different genes that exhibit distinct though overlapping patterns of tissue- and developmental-specific expression. Whereas expression of CD11b and CD11c is almost exclusively restricted to cells of the myeloid lineage, that of CD11a and CD18 is panleukocytic. To begin to understand the mechanisms by which expression of these gene products is restricted to leukocytes and leukocyte subpopulations and to elucidate the mechanisms by which their expression is coordinated, we have cloned and characterized the promoter region of the CD11b gene. A single transcription initiation site has been identified and the region extending 242 base pairs upstream and 71 base pairs downstream of this site has been shown to be sufficient to direct tissue-, cell-, and development-specific expression *in vitro*, which mimics that of the CD11b gene *in vivo*. Within this region there are potential binding sites for transcription factors known to be involved in hematopoietic-specific and phorbol ester-inducible gene expression. Further analysis of this region of the CD11b gene and comparison with the promoters of the CD11a, CD11c, and CD18 genes should lead to significant insights into the molecular mechanisms by which these genes are regulated during hematopoietic development and upon activation.**

CD11/CD18 (leukocyte adhesion molecules) is a family of three surface membrane glycoprotein heterodimers that serves crucial roles in leukocyte adhesion functions (reviewed in ref. 1). Each of the $\alpha$ subunits (CD11a, -b, and -c) is noncovalently linked to a common $\beta$ subunit (CD18). These glycoproteins are members of a larger family of heterodimeric receptors (integrins) mediating specific cell–cell and cell–matrix interactions (2). CD11/CD18 mediate crucial leukocyte functions (e.g., chemotaxis, phagocytosis, aggregation, adhesion to endothelium, and transendothelial migration) *in vitro* and *in vivo* (3, 4). The genes encoding the highly homologous CD11 subunits are located on chromosome 16 p11-p13.1, whereas the CD18 gene has been mapped to chromosome 21 q22 (5). Expression of the CD11 genes occurs in a tissue- and cell-specific manner and responds distinctly to differentiation and environmental stimuli (1). Expression of CD11b and CD11c is limited to myelomonocytic cells, whereas CD11a and CD18 are expressed on all leukocytes. The nonhomologous CD18 gene is concomitantly expressed by the same stimuli that result in the unique and cell-specific expression of the CD11 subunits, thus providing an ideal system for studying coevolution of cis- and trans-acting elements in structurally unrelated genes.

Several disease states caused by or associated with abnormal expression of CD11/CD18 point to the clinical importance of elucidating the molecular mechanisms underlying expression of the genes encoding these adhesion molecules. Inherited lack of expression of CD11/CD18 causes a form of immune deficiency (leukocyte adhesion molecule deficiency) characterized by impaired leukocyte adhesion and recurrent and often fatal bacterial infections (3). Deficient expression of CD11/CD18 on certain lymphomas may contribute to their leukemic transition and escape from immune surveillance (6). In addition, the regions of chromosome 16 and 21 containing the CD11 and CD18 genes, respectively, appear to be involved in a number of the genetic rearrangements frequently observed in patients with various forms of leukemia (7–9), suggesting that abnormal regulation of these genes may contribute to malignant transformation.

To begin a detailed analysis of the regulatory pathways modulating expression of CD11/CD18, we have characterized the promoter region of the myelomonocyte-restricted CD11b gene.*

## MATERIALS AND METHODS

**Isolation of the 5′ Flanking Region of the CD11b Gene.** A 0.1-kilobase (kb) *Xho* I–*Hin*cII fragment derived from the 5′ end of a human CD11b cDNA (10) was used to screen a Charon 21A human chromosome 16-specific genomic library (11). The screening procedure was as described (12), and one positive clone, λCD11b, was isolated.

**Restriction Endonuclease Mapping.** λCD11b DNA was prepared and digested with various restriction endonucleases (New England Biolabs). DNA fragments were subjected to agarose gel electrophoresis and transferred to nitrocellulose (Schleicher & Schuell) as described (13). Prehybridization, hybridization of the 0.1-kb *Xho* I–*Hin*cII cDNA fragment, and washing of filters were as described (14).

**Subcloning of Phage Genomic DNA.** The 5.0-kb *Hin*dIII insert of λCD11b was isolated by electrophoresis followed by electroelution. This fragment was ligated with *Hin*dIII-digested pAA-PZ618 (Gold Biotechnology, St. Louis) to generate the subclone pZ11b 5.0 (Fig. 1).

**DNA Sequencing.** Double-stranded DNA was sequenced by the dideoxynucleotide chain-termination/extension method (15) using Sequenase (United States Biochemical). The extreme 5′ end of the insert of pZ11b 5.0 was sequenced using the universal M13 sequencing primer (New England Biolabs). A series of CD11b-specific oligonucleotide primers were synthesized by Oligos Etc. (Guilford, CT) and used to sequence the remaining areas of the first exon and 5′ flanking region contained in pZ11b 5.0. The entire nucleotide se-

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Abbreviation: PMA, phorbol 12-myristate 13-acetate.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M76724).

quence of this region of pZ11b 5.0 was determined on both DNA strands.

**Computer Analysis.** Sequence analysis was carried out using the Genetics Computer Group Sequence analysis software package (16). Searches against the GenBank data base were performed using the FASTA program (17).

**S1 Nuclease Protection Analysis.** The transcription initiation site of the CD11b gene was identified by S1 nuclease protection analysis using a 332-base-pair (bp) *Nde* I–*Hin*cII fragment containing the 5′ end of the first exon and 5′ flanking sequence. The 5′ end of the noncoding strand was radiolabeled using polynucleotide kinase (New England Biolabs) and [$\gamma$-$^{32}$P]ATP (Amersham). S1 nuclease protection analysis was performed as described (18) using 10 $\mu$g of total RNA isolated (19) from human monocytes (20).

**Cell Culture.** U937 human monocytic leukemia cells, K562 human chronic myelogenous leukemia cells, the Burkitt lymphoma B-lymphoblastoid cell line Daudi, the T-lymphoblastoid cell line CEM, and HeLa human cervical epithelial carcinoma cells were purchased from the American Type Culture Collection (ATCC). Jurkat human leukemic T cells and the JY B-cell line were provided by Tom Wileman (Dana–Farber Cancer Institute, Boston) and Roy Lobb (Biogen), respectively.

**Expression Plasmid Construction.** Promoter activity was assessed using the expression vector poLUC/T1 (Fig. 4), a promoterless plasmid derived from poLUC (provided by J. Habener, Massachusetts General Hospital), which contained a firefly luciferase reporter gene. The luciferase cDNA in poLUC was originally derived from pSVOALΔ5′ (21, 22). poLUC/T1 was generated by replacing a 1.3-kb *Pvu* I–*Hin*dIII fragment of poLUC, which mapped upstream of the reporter gene, with a 0.8-kb *Pvu* I–*Hin*dIII fragment of pKK232-8 (Pharmacia LKB) containing the rRNA transcription terminator T1 (Fig. 4). The polymerase chain reaction was used to generate three fragments of the CD11b gene from pZ11b 5.0, representing nucleotides −775 to +71, −521 to +71, and −242 to +71 relative to the transcription initiation site. These fragments were then subcloned into the *Hin*dIII site of poLUC/T1, which had been "filled-in" using the large Klenow fragment of *Escherichia coli* DNA polymerase I, to generate pLbI, pLbII, and pLbIII, respectively (Fig. 4). A positive control plasmid, poLUCSV/T1, was generated by inserting into the *Sma* I site of poLUC/T1 the 346-bp "filled-in" *Pvu* I–*Hin*dIII fragment of pCH110 (Pharmacia LKB) containing the enhancer and early promoter of simian virus 40 (SV40). The orientations relative to the luciferase gene of the SV40 fragment in poLUCSV/T1 and the CD11b fragments in pLbI–pLbIII were verified by restriction mapping. A negative control plasmid, poLUCH-/T1, was generated by digesting poLUC/T1 with *Hin*dIII, "filling-in," and religation. All expression constructs were verified by DNA sequencing and purified by cesium chloride gradient centrifugation prior to transfection.

**Transfection.** Transfections were performed by electroporation according to Potter *et al.* (23) in nonsupplemented medium at 300 V and 1750 $\mu$F using a Cell-Zap II electroporator (Anderson Electronics, Cambridge, MA) and Gene-Pulser cuvettes (Bio-Rad). Cells were washed once with medium and resuspended at room temperature to a concentration of $3 \times 10^7$ cells per ml. One milliliter of cells was transfected with 40 $\mu$g of luciferase test plasmid together with 20 $\mu$g of the plasmid pCH110 which contains the *lacZ* gene. Electroporated U937 and CEM cells were equally divided and either not treated or treated for 16 hr with phorbol 12-myristate 13-acetate (PMA; Sigma) at 100 ng/ml prior to harvesting.

**Luciferase and β-Galactosidase Assays.** Sixteen hours after electroporation, cells were pelleted and washed twice with Dulbecco's phosphate-buffered saline (D-PBS). The cell pellet was then resuspended in 1 ml of D-PBS, and one-half was assayed for β-galactosidase activity and the other was assayed for luciferase activity using the respective assay systems purchased from Promega. Luciferase activity, assessed as light output, was measured using a LKB model 1251 Luminometer integrating peak luminescence 1–16 s after injection of assay buffer.

**RNA Blot Analysis.** Total RNA for Northern blot or slot blot analysis (14) was isolated from untreated U937 and Jurkat cells or from cells treated with PMA for various times or electroporated 16 hr earlier in the presence of 40 $\mu$g of poLUCH-/T1 and 20 $\mu$g of pCH110. Blotted RNA was hybridized with a $^{32}$P-labeled *Pst* I–*Pvu* I fragment of a CD11b cDNA (10) and subsequently with β-tubulin cDNA (ATCC no. 57105).

## RESULTS AND DISCUSSION

**Isolation of the 5′ Flanking Region of the CD11b Gene.** A human genomic library was screened with an *Xho* I–*Hin*cII restriction fragment containing the extreme 5′ end of a CD11b cDNA clone (10). Approximately $10^5$ recombinants were screened, and one reactive clone, designated λCD11b, was isolated. The 5.0-kb *Hin*dIII insert of λCD11b was subcloned into pAA-PZ618 to generate pZ11b 5.0. The strategy used to sequence the 1.8 kb of pZ11b 5.0 that contains the first exon and immediate 5′ flanking region of the CD11b gene is illustrated in Fig. 1; the sequence is shown in Fig. 2.

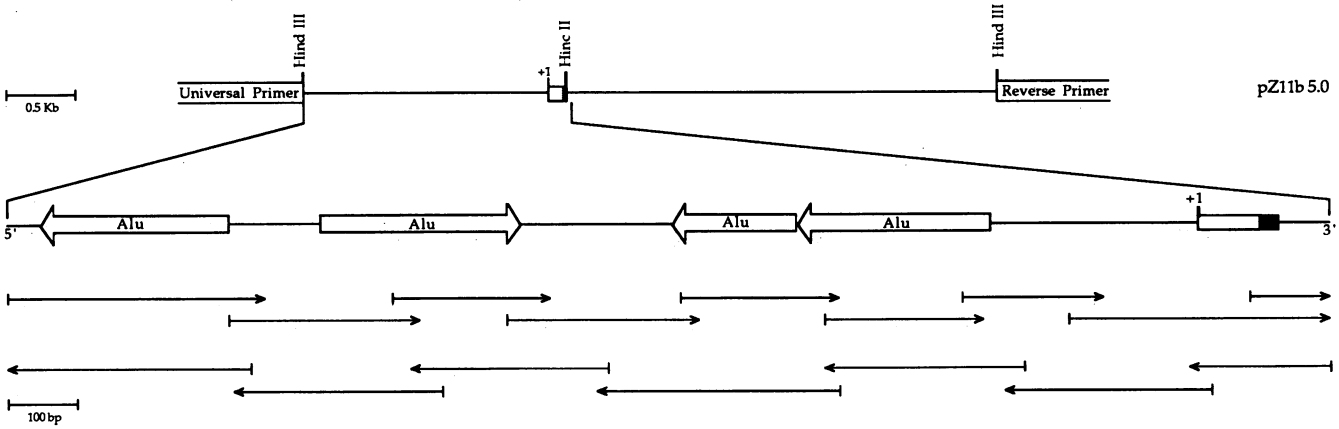


FIG. 1. Partial restriction map of pZ11b 5.0 and the relative position of exon 1 of the CD11b gene (boxed, with the filled portion representing the coding region). The sequenced region of pZ11b 5.0 is expanded to show the positions and orientations of the *Alu* repeats. The sequencing strategy is depicted by arrows denoting the length and direction of individual sequencing reactions.

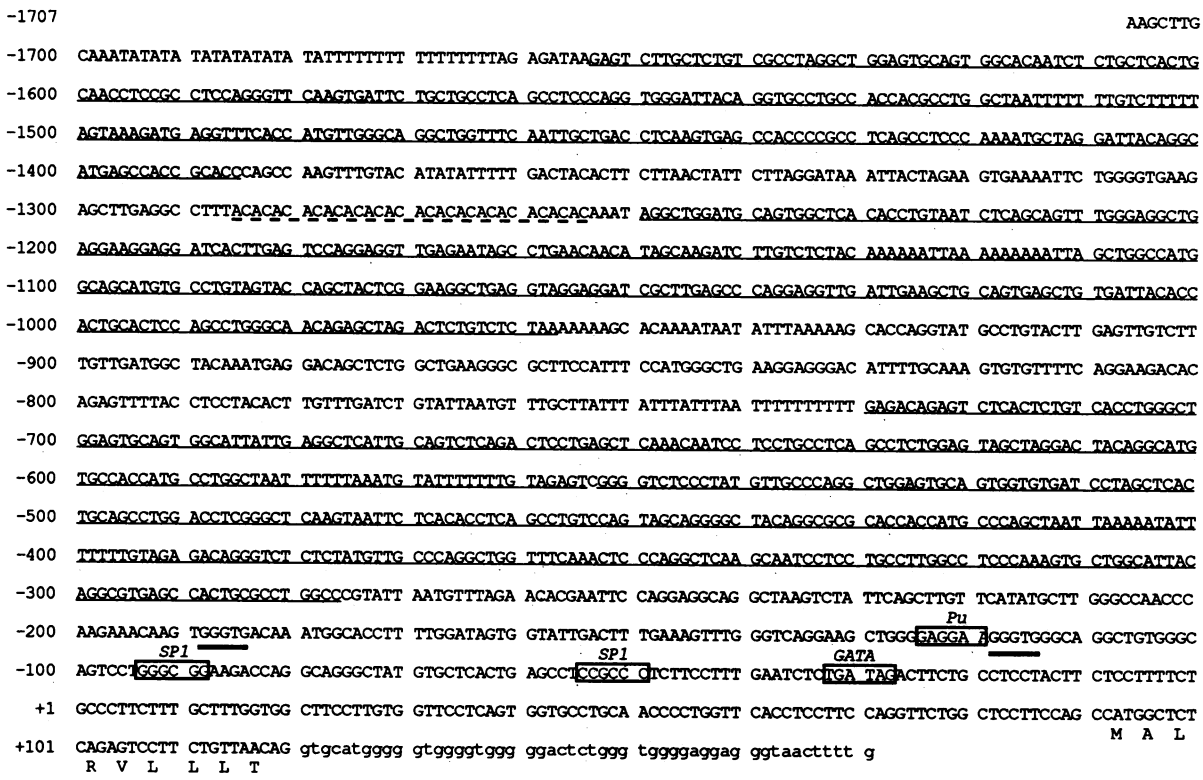


FIG. 2. Nucleotide sequence of the 5′ end of the human CD11b gene. Exon 1 begins at the transcription initiation site (+1) and ends at +120, after which intron 1 is depicted in lowercase lettering. Below the coding portion of exon 1, the derived CD11b amino acid sequence is indicated in single-letter code (10). Potential binding sites for transcription factors Sp1, GATA, and PU-1 are boxed and *Alu* sequences are underlined. The repeat element $(AC)_{16}$ is indicated by an interrupted underline. The complementary sequences of the two CACCC motifs are marked by heavy underlines.

**Transcription Initiation Site and First Exon.** S1 nuclease protection analysis established that transcription is initiated at a single site (Fig. 3), the guanine at nucleotide position 1 (Fig. 2). Comparison of the CD11b genomic and cDNA sequences indicates that the first exon of the CD11b gene consists of 120 bp composed of 92 bp of 5′ noncoding sequence and 28 bp encoding the first nine amino acids of the CD11b leader polypeptide. The first intron of the CD11b gene interrupts the codon specifying the 10th amino acid of the leader polypeptide after the first nucleotide of the triplet (Fig. 2). The 5′ end of this intron is in general agreement with the consensus sequence derived from the equivalent position in other eukaryotic genes (24). The structures of the genes encoding CD11b, CD11c, and CD11a are likely to be similar since they probably arose from a common ancestor by gene duplication (10, 25). Recently the structure of the CD11c gene has been determined (26) and, although the first exon and 5′ flanking sequence were not isolated, the first intron was shown to interrupt the 5′ noncoding region and the second to interrupt the codon specifying the 13th amino acid of the leader polypeptide. Therefore, the second intron of the CD11c gene appears analogous to the first intron of the CD11b gene, which may have lost an intron in its 5′ noncoding region.

**5′ Flanking Region.** Upstream from the transcription initiation site, 1707 nucleotides have been sequenced (Fig. 2). Fifty-nine percent of this sequence consists of four *Alu* repeats. One, between nucleotides −1250 and −958, is complete and in the same orientation as the CD11b gene; two, one between nucleotides −1654 and −1386 and the other between −553 and −277, are complete and inverted with respect to the CD11b gene, and nucleotides −730 to −555 represent the 3′ half of an inverted *Alu* sequence. Between nucleotides −1286 and −1255 there is the alternating purine/pyrimidine sequence, $(AC)_{16}$, capable, in theory, of forming Z-DNA. Alternating AC sequences may act as signals for gene conversion and may constitute "hot spots" of recombination and/or gene rearrangement (27). Such elements may also weakly inhibit or enhance transcription (28, 29). Since the CD11b gene probably arose by gene duplication, it is possible that Z-DNA sequences may have played a role in this process. In addition, the unusually large number of *Alu* sequences in the 5′ flanking region of the CD11b gene suggests that retroposition might also have been involved.

**Tissue-, Cell-, and Development-Specific Expression Directed by the Promoter Region.** To analyze the 5′ flanking region of the CD11b gene for promoter activity, three constructs were generated, pLbI, pLbII, and pLbIII (Fig. 4). These contained 775, 521, and 242 bp, respectively, of 5′ flanking region together with the 71 bp at the 5′ end of the first exon cloned into the plasmid poLUC/T1 upstream of the firefly luciferase gene. These constructs were transfected into undifferentiated U937 cells and U937 cells induced with 100 ng of PMA per ml to differentiate along the monocytic pathway. At this concentration, PMA induced CD11b mRNA in U937 within 8 hr, as determined by Northern blot analysis (data not shown). In undifferentiated U937 cells, the three constructs resulted in a mean luciferase activity 33-fold above the background level conferred by the promoterless construct poLUCH-/T1 (Fig. 5). In PMA-induced U937 cells, the luciferase activity conferred by each construct increased an average of 3-fold to 105-fold above background (Fig. 5). The transcribed 71 nucleotides of the CD11b gene included in the expression plasmids have none of the sequences implicated in mediating mRNA stability (30–32), suggesting that the increase in luciferase activity that accompanied U937 differentiation is due to an increase in transcription directed by the region extending 242 bp upstream of the transcription initi-

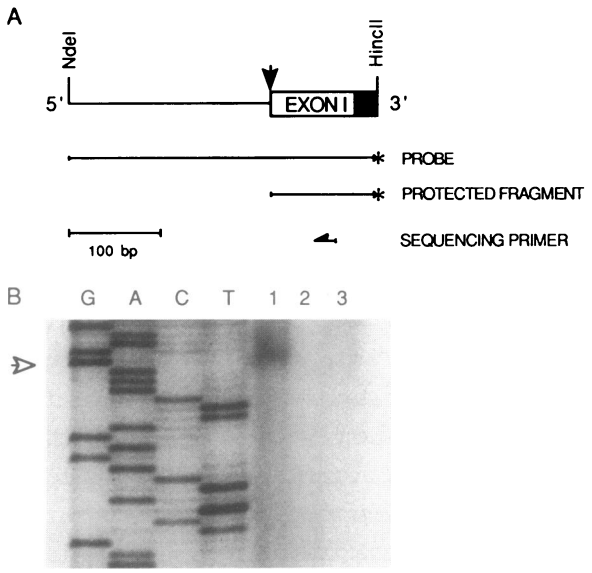


FIG. 3. Identification of the transcription initiation site of the CD11b gene. (*A*) The 5′ region of the CD11b gene is represented at the top; the open and filled boxes indicate the noncoding and coding regions, respectively, of exon 1 and the line represents 5′ flanking sequence. The arrow marks the transcription initiation site identified by S1 nuclease protection analysis. The *Nde* I–*Hin*cII genomic DNA probe is represented below as is the fragment protected from S1 nuclease digestion. The primer used to generate the reference sequencing reactions of the CD11b gene is also indicated; its 5′ end is shifted 44 nucleotides upstream of the *Hin*cII cleavage site. (*B*) Lane 1, product of protection of the *Nde* I–*Hin*cII fragment from S1 nuclease digestion using 10 μg of total human monocyte RNA; lane 2, product of protection using 10 μg of yeast tRNA; lane 3, undigested *Nde* I–*Hin*cII fragment used as probe. Lanes marked T, C, A, and G represent the reference dideoxy sequencing reactions of the noncoding strand of the CD11b gene. The arrow marks the 115-nucleotide fragment protected from S1 nuclease digestion, which in the coding strand aligns with the C residue at nucleotide +44. Accounting for the 44-nucleotide displacement of the 5′ ends of the reference sequencing primer and S1 nuclease probe, this analysis places the initiation site of the CD11b gene at a G residue (+1 in Fig. 2).

ation site. To demonstrate that the effect of PMA on transcription of pLbI, pLbII, and pLbIII in U937 cells was due

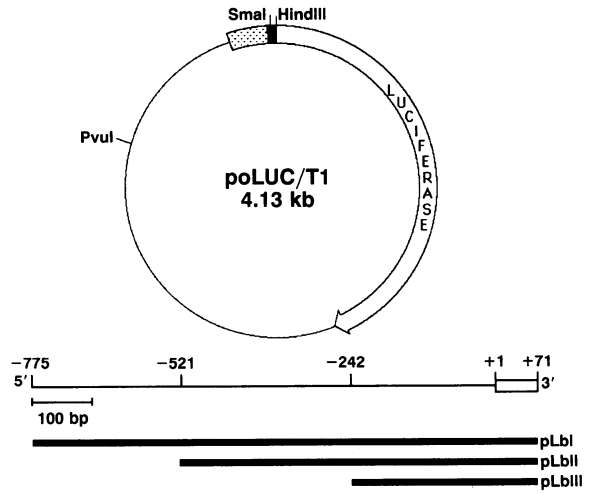


FIG. 4. Schematic of the plasmid poLUC/T1 and the three CD11b gene-derived constructs, pLbI, pLbII, and pLbIII. Positions of the ribosomal transcription terminator T1 (stippled box), the multiple cloning site (filled box), and the *Pvu* I and *Hin*dIII sites used in the construction of poLUC/T1 are marked. Also indicated is the *Sma* I site used to construct poLUCSV/T1 and the relative position of the luciferase gene.

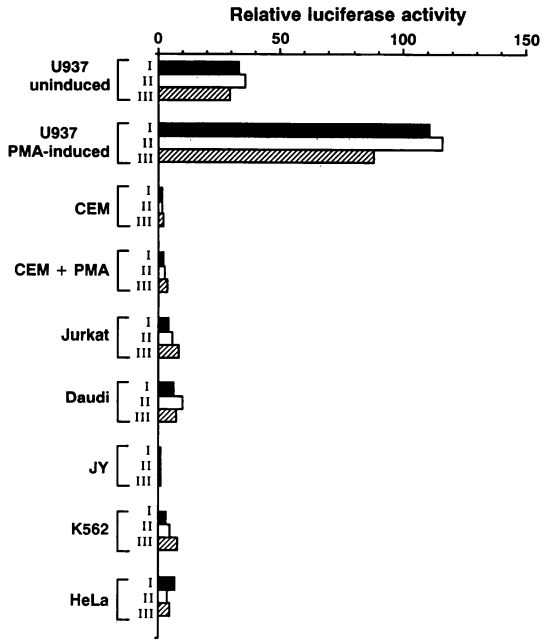


FIG. 5. Relative luciferase activity in various cell types directed by the expression constructs pLbI–pLbIII. Transfections of pLb plasmids were done in parallel with transfections of the positive control plasmid poLUCSV/T1, the negative control plasmid poLUCH-/T1, and cells electroporated with no DNA. All luciferase constructs were cotransfected with pCH110. Transfected cells were examined for luciferase and β-galactosidase activity 16 hr later. Relative luciferase activity is expressed as fold increases in light units above that resulting from poLUCH-/T1 transfection, normalized for β-galactosidase activity.

to induction of monocytic differentiation and not to PMA *per se*, these constructs were transfected into untreated or PMA-treated CEM cells. PMA had little effect on the level of transcription directed by the expression constructs in CEM cells (Fig. 5). Such a low level of activity would be expected in a cell type in which CD11b is not detected. Transfections performed in other leukocyte cell lines that do not normally express CD11b revealed levels of expression equal to or less than those detected in the nonleukocytic cell line HeLa (Fig. 5). The level of transcription directed by these constructs in uninduced U937 cells, which do not normally express CD11b (Fig. 5), appears to reflect the degree to which the CD11b gene is activated by the electroporation procedure (Fig. 6).

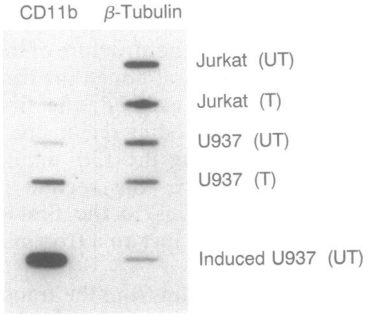


FIG. 6. Effect of electroporation on CD11b expression. Ten micrograms of total RNA from untransfected Jurkat or U937 cells (UT) or from cells transfected (T) by electroporation 16 hr earlier with poLUCH-/T1 and pCH110 was slot-blotted onto nitrocellulose and hybridized with radiolabeled CD11b cDNA. Blots were subsequently stripped of radiolabel and rehybridized with a radiolabeled β-tubulin-specific probe. Shown for comparison is the signal observed with 5 μg of total RNA derived from U937 cells induced for 48 hr with PMA (100 ng/ml).

This phenomenon may also explain why the levels of luciferase activity conferred by pLbI, pLbII, and pLbIII are above background in other CD11b-negative cell lines (Fig. 6). Nevertheless, these results indicate that the region of the CD11b gene extending 242 bp upstream and 71 bp downstream of the transcription initiation site is sufficient to direct transcription *in vitro* that mirrors the tissue-, cell-, and development-specific expression of the gene *in vivo*. The 242-bp region contains two potential Sp1 binding sites, one in the coding strand beginning at nucleotide −94 and one in the noncoding strand beginning at nucleotide −50. Between nucleotides −125 and −120 there is the sequence GAGGAA, which represents a potential binding site for the transcription factor PU-1 (33). Since this factor appears macrophage and B-cell specific (33), it may be involved in the tissue-specific expression of the CD11b gene. Starting at nucleotide −33 in the coding strand there is the sequence TGATAG, which represents a potential binding site for the "GATA" family of transcription activators (34). Members of this family, GATA-1 and GATA-3, are lineage-specific hematopoietic transcription factors that have been shown to regulate erythroid (35) and T-cell-specific (36, 37) genes, respectively. In the noncoding strand beginning at nucleotides −115 and −185 there are two CACCC motifs that, although originally defined as important functional features of β-globin gene promoters (38), have also been shown to be important for the activity of nonglobin promoters (39, 40). Phorbol ester induction of gene transcription involves the activation of cis-acting elements including NF-κB (41), AP-1 (42, 43), AP-2 (44, 45), and AP-3 (46). Within the 242-bp region, no potential NF-κB sites were identified but sequences resembling AP-1, AP-2, and AP-3 elements are present. In the noncoding strand, beginning at nucleotide −135, there is the sequence CTGACCCA similar to the AP-1 consensus sequence $\begin{matrix}C\\G\end{matrix}TGACT\begin{matrix}C\\A\end{matrix}A$ (47). There are eight sequences with one-residue mismatches to the AP-2 consensus sequence (45). Two, one in each strand, overlap between −214 and −201, five overlap in the noncoding strand between −120 and −89, and one, again in the noncoding strand, is present between −81 and −72. There are two overlapping sequences on the coding strand between −71 and −57 that have single residue mismatches to the AP-3 consensus sequence (46). A more precise characterization of the cis-acting elements in this region and identification of the trans-acting factors with which they interact should increase our understanding of how transcription of myelomonocyte-restricted genes is regulated. In addition, these studies should allow comparative analyses with other members of the integrin family.

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