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Biochemistry

CD18 (β_2 leukocyte integrin) promoter requires PU.1 transcription factor for myeloid activity

(gene expression regulation/monocytes)

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ABSTRACT **Normal cellular differentiation is linked to tightly regulated gene transcription. However, the DNA elements and trans-acting factors that regulate transcription in myeloid cells are poorly defined. CD18, the β chain of the leukocyte integrins, is transcriptionally regulated during myeloid differentiation. The CD18 promoter is active after transfection into myeloid cells. We demonstrate that a region of the CD18 promoter that contains two binding sites for the PU.1 transcription factor is required for activity in myeloid cells. These sites are bound by *in vitro* translated PU.1 and by PU.1 from myeloid nuclear extracts. Mutagenesis of these sites abrogates binding by PU.1 and substantially decreases promoter activity in myeloid cells. Thus, the leukocyte-specific transcription factor PU.1 is required for myeloid activity of CD18.**

Monocytes and granulocytes, which are collectively referred to as myeloid cells, play critical roles in the immune and inflammatory responses. These mature hematopoietic cells differentiate from pluripotent bone marrow precursors under the influence of cytokines and other regulatory molecules. Cellular differentiation is linked to tightly regulated gene expression, and such regulation is frequently controlled at the level of transcription (1). Acute leukemia is a clinical manifestation of disordered hematopoietic differentiation; some types of acute leukemia are caused by abnormalities of transcription factors.

Little is known of the sequence-specific DNA motifs and the trans-acting factors that regulate transcription in normal myeloid differentiation. PU.1, a member of the *ets* family of transcription factors, is expressed by B lymphocytes and monocyte/macrophages. It binds to DNA in a sequence-specific manner and transcriptionally regulates genes whose regulatory elements contain the PU.1 cognate binding site (2). A functional PU.1 site is present in the promoter of the gene encoding CD11b, a gene that is expressed predominantly by myeloid cells (3, 4).

CD18 (β_2 leukocyte integrin) is expressed exclusively by lymphocytes and myeloid cells. It forms a heterodimer with either CD11a, CD11b, or CD11c to generate antigens commonly known as LFA-1, Mo-1 (Mac-1), and p150/95, respectively. Failure to express adequate CD18 is the cause of leukocyte adhesion deficiency, a congenital immunodeficiency in which defective leukocyte function leads to recurrent infections and premature death (5). During myeloid differentiation, CD18 mRNA expression increases markedly (6), due to an increase in CD18 transcription (7, 8). The transfected CD18 promoter directs expression of a linked reporter gene in myeloid cells, and its activity increases with myeloid differentiation (7).

In this report, we localize the myeloid activity of the CD18 promoter to a region that contains two binding sites for the PU.1 transcription factor.§ Mutagenesis of these sites significantly reduces activity of the CD18 promoter and causes a corresponding decrease in binding by PU.1. Thus, the CD18 promoter requires binding by the PU.1 transcription factor for myeloid expression.

MATERIALS AND METHODS

Transfection. About 5×10^6 logarithmically growing U-937 (ATCC no. CRL 1593) cells are electroporated, as described (7), with 20 μg of CD18 promoter/luc constructs and 1 μg of cytomegalovirus promoter/human growth hormone construct. Luciferase activity is normalized to the human growth hormone internal control (Allegro, Nichols Institute, San Juan Capistrano, CA) to obtain normalized relative light units (NRLU); activity of deletion and mutation constructs is expressed relative to the full-length promoter. Transfection results represent the data from at least three separate experiments.

CD18 Promoter Deletions and Mutations. Promoter deletion constructs were prepared by exonuclease III digestion (9) of CD18(-0.9)/luc or by a modification of PCR. Oligonucleotides were synthesized with a *Sac* I restriction site linked to the desired 5′ terminus of the CD18 promoter. PCR was done with the 5′ oligonucleotide and a luciferase antisense oligonucleotide (CTCTAGAGGATAGAATGGCG), using construct CD18(-0.9)/luc as a template. Thirty-five cycles of 94°C × 1.5 min, 52°C × 1.5 min, and 72°C × 1.5 min were followed by 72°C × 7 min. The amplified products were digested with *Sac* I and *Hin*dIII and ligated to *Sac* I/*Hin*dIII-digested pXP1ΔBam (10). For PCR-based mutagenesis, 5′ oligonucleotides incorporate the desired mutation (underlined in the sequences below). All constructs were confirmed by dideoxynucleotide chain-termination sequencing with Sequenase (United States Biochemical).

The following oligonucleotides, prepared by phosphoramidite chemistry on an Applied Biosystems model 381A DNA synthesizer, were used to generate CD18 deletions and mutations: −148 (GCGGAGCTCTCTGACATCAGAGC); −120 (GCGGAGCTCGGAGAGGGGCAGGGGTGA); −96 (GCGGAGCTCTCTGACATCAGAGC); −79 (GCGGAGCTCCACTTCCTCCAAGGAGG); −72 (GCGGAGCTCCAAGGAGGAGCTGAGA); −40 (GCGGAGCTCAGGACTTCACGACCCG); −79 m-dist PU.1 (GCGGAGCTCCACTTGGTCCAAGGAGGAGCT); −72 m-prox PU.1 (GCGGAGCTCCAAGGAGGAGCTGAGACCAACAGGAA). Note that the proximal PU.1 site (prox PU.1) contains the consensus GAGGAA on the top strand, whereas the distal PU.1 site (dist PU.1) contains the

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Abbreviations: PMA, phorbol 12-myristate 13-acetate; NRLU, normalized relative light units.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M77675).

GAGGAA on the bottom strand. The −79 m-prox PU.1 was prepared by subcloning the mutagenized region of −72 m-prox PU.1, from a *Sty* I site at −69 to the *Hin*dIII site in the polylinker, into the −79 construct. The −79 mm-PU.1 was prepared by subcloning the mutagenized region of −72 m-prox PU.1, from a *Sty* I site at −69 to the *Hin*dIII site in the polylinker, into −79 m-dist PU.1.

Preparation of Nuclear Extracts and *in Vitro* Translated Protein. Nuclear extracts were prepared by a modification of the technique of Dignam (11). Soluble nuclear proteins were extracted for 30 min at 4°C in buffer C and dialyzed against buffer D at 4°C for 2 hr in a Spectra/Por microdialyzer (Spectrum Laboratories, Houston). Buffers A and C were supplemented with freshly added antipain, aprotinin, leupeptin, pepstatin, soybean trypsin-chymotrypsin inhibitor (all 5 μg/ml), 0.1 mM benzamidine, 0.5 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride (all from Sigma). Buffer D was supplemented with 0.5 mM dithiothreitol and 0.5 mM phenylmethylsulfonyl fluoride. Protein concentration was determined by the Pierce BCA protein assay, and samples were frozen in liquid nitrogen and stored at −80°C.

In vitro translated PU.1 protein was prepared by using 1 μg of supercoiled PU.1 KS+ (2) in the TNT T3 coupled reticulocyte lysate system (Promega); unprogrammed reticulocyte lysate was prepared similarly but without DNA addition.

DNase I Footprinting. An oligonucleotide corresponding to +28/+9 of CD18 antisense sequence (TTTGCTACCAGTCTGCCCTG) (7) was 5′ end-labeled with polynucleotide kinase (New England Biolabs) and [γ-^{32}P]ATP, and heated to 65°C for 10 min to inactivate the kinase. PCR was performed with the −148 oligonucleotide described above and a 1.3-kb *Ava* I fragment that overlaps the CD18 promoter as template (7). Amplified products were recovered by the crush and soak method (12) from a 4% polyacrylamide gel in 0.5× TBE (1× TBE is 90 mM Tris/90 mM boric acid/2 mM EDTA, pH 8.3).

Nuclear extracts were equilibrated in 10 mM Tris, pH 7.4/40 mM KCl/1 mM EDTA/1% Ficoll/1 mM 2-mercaptoethanol for 10 min at room temperature and then incubated with 5000 cpm of end-labeled footprinting probe for 30 min at room temperature. Samples were digested with DNase I (Sigma) at 2 μg/ml and room temperature for 1 min, phenol-extracted, and ethanol-precipitated. Samples without nuclear extracts were digested with DNase I (0.5 μg/ml) at room temperature for 20 sec. One half of the resultant products were electrophoresed in a 6% polyacrylamide/7 M urea gel alongside the same probes subjected to chemical sequencing (12), and autoradiography was done.

Electrophoretic Mobility Shift Assay. Complementary oligonucleotides, corresponding to −85/−37 of the CD18 promoter (see Fig. 4*A* for sequence), were annealed and 5′ end-labeled with polynucleotide kinase and [γ-^{32}P]ATP (ICN). The mutagenized −85/−37 probe contains GG → CC mutations in the two PU.1 sites. Probes corresponding to −89/−58 (dist PU.1) and −59/−31 (prox PU.1) were annealed, and overhanging ends were labeled by the Klenow fragment of DNA polymerase I and [α-^{32}P]dCTP (ICN). Radiolabeled probe (0.1 ng) was incubated for 10 min on ice with 1 μl of unprogrammed reticulocyte lysate, 1 μl of *in vitro* translated PU.1, or 10 μg of nuclear extract in a 15-μl reaction containing 10 mM Tris, pH 7.5/50 mM NaCl/1 mM EDTA/1 mM β-mercaptoethanol/1% Ficoll/2.5 μg of poly(dI·dC) (Pharmacia). Where indicated, a 100-fold molar excess of unlabeled homologous probe or a nonspecific competitor from an irrelevant region of the CD18 (−903/−883; TTGCAGTGAGCTGAGATCACG) was added as competitor before the addition of the radiolabeled probe. Products were electrophoresed at 150 V in a 5% acrylamide/bis, 19:1, gel in 0.5× TBE before autoradiography.

RESULTS

Localization of a Positive Regulatory CD18 DNA Element. We have previously shown that the CD18 promoter directs expression of the linked luciferase reporter gene after transient transfection into U-937 cells (7). To localize DNA elements that direct CD18 promoter myeloid activity, we transfected CD18 promoter deletion constructs into U-937 cells (Fig. 1). Deletion to 79 nt from the transcriptional start site did not significantly affect promoter activity. However, deletion to −72 and to −40 resulted in a dramatic loss of promoter activity. Thus, the region within 79 nt of the CD18 transcriptional start site is sufficient to direct myeloid expression of a linked reporter gene.

PU.1 Sites Are Required for CD18 Expression by Myeloid Cells. PU.1 is a member of the *ets* family of transcription factors that is expressed by B lymphocytes and monocyte/macrophages (2). Because PU.1, like CD18, is expressed by leukocytes, it is a candidate for a transcription factor that regulates CD18 expression.

Two consensus PU.1-binding sites are present in the 79-nt region of the CD18 promoter, which directs myeloid activity. The GAGGAA sequence is on the top strand at −55 (proximal site), whereas it is on the bottom strand at −70 (distal site). We mutagenized (GAGGAA → GACCAA) in the distal (−79 m-dist PU.1) and proximal (−79 m-prox PU.1) PU.1 sites individually, and together (−79 mm-PU.1), to determine their role in the myeloid activity of the CD18 promoter. Fig. 2 indicates that transfection into U-937 cells of constructs containing mutations at either PU.1 site significantly reduces CD18 promoter activity. Mutation of both PU.1 sites results in negligible expression, comparable with that of the −40 construct. This result suggests that both PU.1 sites are required for full promoter activity, and they may function in a cooperative manner.

Myeloid Proteins Bind to the CD18 PU.1 Sites. We used DNase I footprinting to determine whether myeloid nuclear proteins bind to the region of the CD18 promoter that is required for myeloid activity. Compared with the probe in the absence of nuclear extract (naked DNA, lane N in Fig. 3), U-937 nuclear extract (lane U) protects from DNase I digestion a 17- to 20-nt region that contains the proximal PU.1 site (vertical arrow) and a downstream region (vertical bar). Immediately 3′ to the GAGGAA sequence of the proximal PU.1 site is a prominent hypersensitive band (indicated by *); a similar footprint is seen over the distal PU.1 site.

CD18 Promoter Is Bound by PU.1 from Myeloid Cells. Electrophoretic mobility shift assay was done with a radiolabeled, double-stranded oligonucleotide that corresponds to −85/−37 of the CD18 promoter, a region that contains both potential PU.1 sites (Fig. 4*A*). The probe is bound by *in vitro* translated PU.1 (Fig. 4*B*, lane 3; filled arrow) but not by

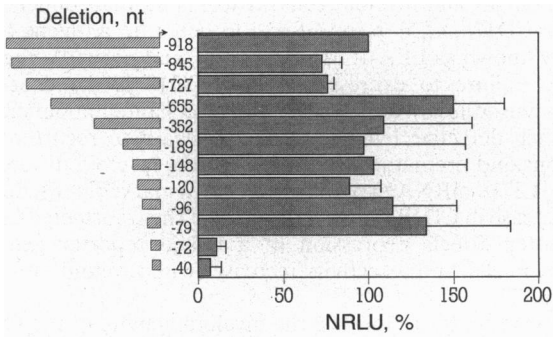

FIG. 1. Deletion analysis of CD18 promoter. Deletion constructs are displayed schematically at left. The corresponding mean and SEM of normalized relative light units (NRLU) relative to the full-length CD18 promoter are displayed at right. Data represent results from at least three independent transfections into uninduced U-937 cells.

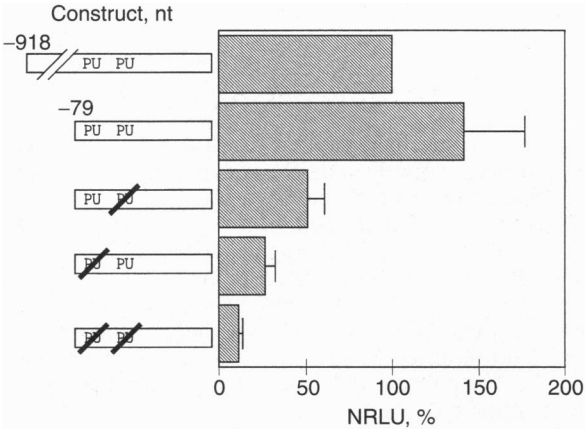

FIG. 2. Mutagenesis of CD18 PU.1 sites. Mutagenized CD18 PU.1 sites (GAGGAA → GACCAA) are displayed schematically at left. Mean activity and SEM are expressed relative to the full-length promoter. Data represent results from at least three independent transfections into uninduced U-937 cells.

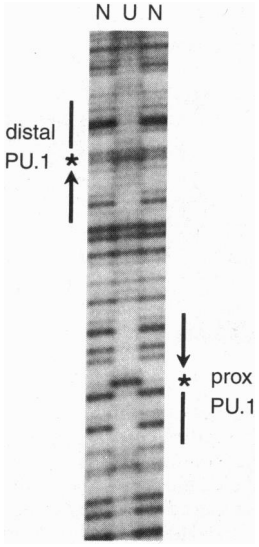

FIG. 3. DNase I footprinting. A probe corresponding to nt −148/+28 relative to the CD18 transcriptional start site was radiolabeled with ^{32}P on the bottom strand. Compared with the probe in the absence of nuclear extract (naked DNA, N lanes), 10 μg of U-937 nuclear extract (U lane) protects both PU.1 sites from DNase I digestion (vertical arrows and bars). Locations and orientations of the proximal and distal PU.1 sites (GAGGAA) are indicated by arrows, and DNase I hypersensitive sites are indicated by stars.

unprogrammed reticulocyte lysate (lane 2). PU.1 binding is abrogated by a 100-fold molar excess of the unlabeled homologous probe (lane 4) but not by a nonspecific probe (lane 5). Nuclear extract from U-937 cells binds to the probe to generate a retarded band with an electrophoretic mobility similar to that of the *in vitro*-translated PU.1 protein (lane 8). Abrogation of this species by a molar excess of unlabeled specific probe (lane 9), but not by nonspecific probe (lane 10), demonstrates its binding specificity.

We confirmed that the myeloid-binding species that comigrates with *in vitro* translated PU.1 indeed represents PU.1. Preincubation of *in vitro* translated PU.1 with α PU.1 antibody abrogates binding and results in a supershifted complex (lane 6, open arrow). No such abrogation of binding or supershift is seen with preimmune serum (lane 7). Preincubation of U-937 nuclear extract with α PU.1 antibody abrogates binding of the comigrating species (lane 11; the supershifted complex is not visible because it comigrates with other binding proteins). This complex is not altered by preimmune serum (lane 12).

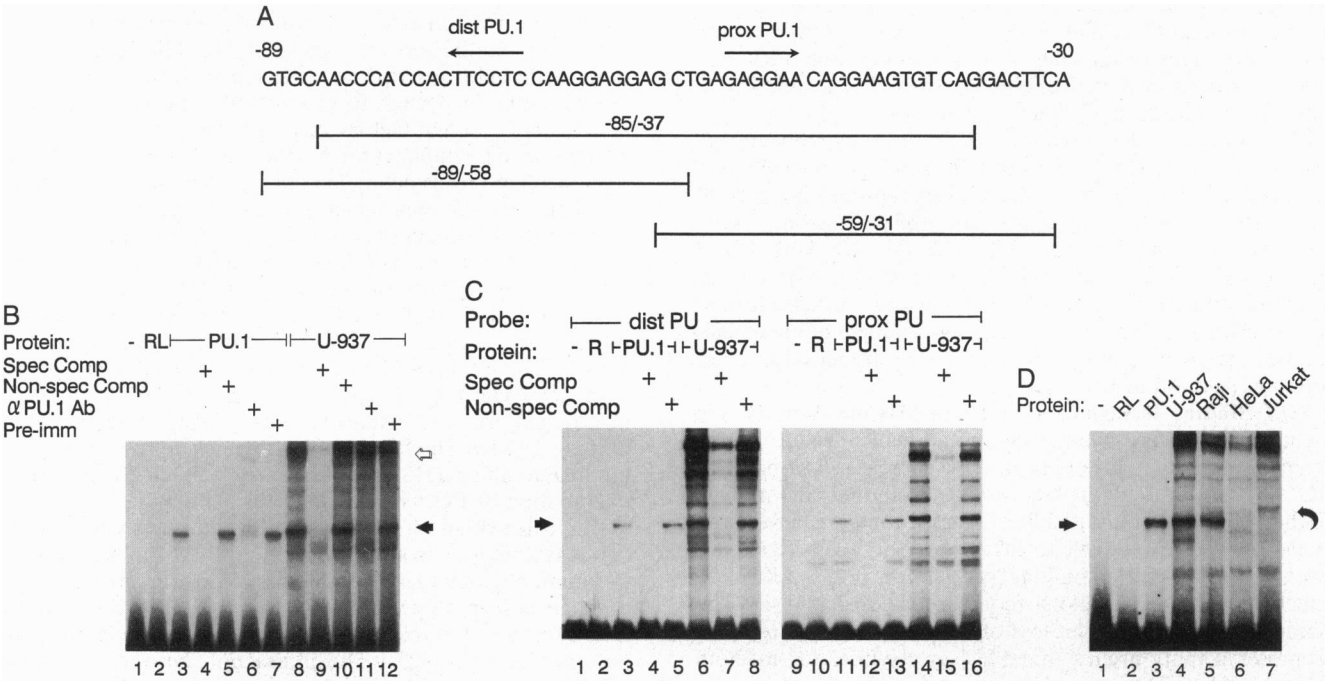

FIG. 4. Electrophoretic mobility shift assay. (*A*) Oligonucleotide probes are schematized below CD18 promoter sequence. Orientations of the PU.1 sequences are indicated by arrows. (*B*) Radiolabeled CD18 −85/−37 probe was electrophoresed in a 5% nondenaturing polyacrylamide gel/0.5× TBE in the absence of added protein (lane 1) or after incubation with 1 μl of unprogrammed reticulocyte lysate (lane 2; labeled RL), 1 μl of *in vitro*-translated PU.1 (lanes 3–7), or 10 μg of nuclear extract from U-937 myeloid cells (lanes 8–12). Specificity of PU.1 binding is demonstrated by competition (Comp) with 100-fold molar excess of either unlabeled −85/−37 probe (Spec probe, lanes 4 and 9) or irrelevant probe (Non-spec; lanes 5 and 10). Filled arrow indicates the location of probe bound by PU.1. *In vitro* translated PU.1 and PU.1 from U-937 nuclear extract are supershifted by α PU.1 antibody (lanes 6 and 11, respectively; open arrow) but not preimmune serum (Pre-imm; lanes 7 and 12). (*C*) PU.1 binds to each of the PU.1 sites independently. *In vitro* translated PU.1 (lanes 3–5, 11–13; left arrow) and PU.1 from U-937 nuclear extract (lanes 6–8, 14–16) but not unprogrammed reticulocyte lysate (R; lanes 2 and 10) bind in a specific manner to probes containing either the distal PU.1 site (lanes 1–8) or the proximal PU.1 site (lanes 9–16). (*D*) PU.1 binding activity is seen in nuclear extracts from U-937 (myeloid; lane 4, left arrow) and Raji (B lymphocyte; lane 5) cells but not from HeLa (nonhematopoietic; lane 6) or Jurkat (T lymphocyte; lane 7) cells. A unique binding species is present in the Jurkat extract (curved arrow).

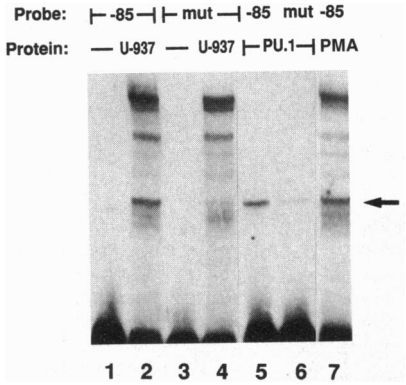

FIG. 5. PU.1 binding site mutations impair PU.1 binding. Electrophoretic mobility shift assay was done with a radiolabeled, −85/−37 probe (labeled −85) and the same probe containing GG → CC mutations in the two PU.1-binding sites (labeled mut). Relative to the wild-type probe, the mutated probe exhibits reduced binding by *in vitro* translated PU.1 (right arrow; compare lanes 5 and 6) and by PU.1 from nuclear extracts of uninduced U-937 cells (compare lanes 2 and 4). PU.1 binding to the −85/−37 probe is not altered after PMA-induced differentiation of U-937 cells (labeled PMA; compare lanes 2 and 7).

Mutagenesis of either PU.1 site significantly reduces CD18 promoter activity (Fig. 2). We sought to determine whether each site is bound by the PU.1 transcription factor. Probes were prepared that include, in isolation, either the distal PU.1 site (dist PU.1, corresponding to −89/−58) or the proximal PU.1 site (prox PU.1, corresponding to −59/−31). Each of the individual PU.1 sites is bound, in a specific manner, by *in vitro* translated PU.1 and by PU.1 from U-937 cells (Fig. 4*C*; left arrow). PU.1 that binds to the individual PU.1 site probes comigrates with PU.1 that binds to the −85/−37 probe (data not shown). This result suggests that although the −85/−37 probe contains two active binding sites, PU.1 binds in a monomeric, rather than dimeric, form.

PU.1 is expressed by monocytic cells and B lymphocytes (2, 13), but not by T lymphocytes or nonhematopoietic cells. We prepared nuclear extracts from cell lines representing each of these tissue types. PU.1 binding to the −85/−37 probe is seen with extracts from monocytic (U-937; Fig. 4*D*, lane 4, left arrow) and B lymphoid cells (Raji; lane 5) but not from nonhematopoietic (HeLa; lane 6) or T-lymphoid cells (Jurkat; lane 7). Although Jurkat cells contain a unique binding species (curved arrow), all other binding species besides PU.1 are common to the four cell types.

PU.1 Binding Is Required for CD18 Myeloid Activity. We sought to determine whether the mutations that reduce CD18 promoter activity in myeloid cells also decrease PU.1 binding. GG → CC mutations in the two PU.1-binding sites reduce binding by *in vitro* translated PU.1 (Fig. 5; compare lanes 5 and 6) and by PU.1 from nuclear extracts of U-937 cells (compare lanes 2 and 4). Other binding species are not significantly affected by these mutations (compare lanes 2 and 4). The direct correlation between loss of PU.1 binding and reduced promoter activity argues that PU.1 is essential for myeloid activity of the CD18 promoter.

PU.1 Binding Does Not Entirely Account for Increased CD18 Transcription During Myeloid Differentiation. Phorbol 12-myristate 13-acetate (PMA) induces greater myeloid differentiation of U-937 cells, increases CD18 transcription (6), and increases expression directed by the CD18 promoter after transfection into U-937 cells (7). We sought to determine whether PMA induction of U-937 cells is accompanied by increased PU.1-binding activity to the CD18 promoter. PU.1 binding to the wild-type −85/−37 probe is not altered after PMA-induced differentiation of U-937 cells (Fig. 5; compare lanes 2 and 7). Therefore, PU.1 binding alone is not sufficient to account for the increase in CD18 promoter activity after PMA-induced myeloid differentiation.

DISCUSSION

The molecular mechanisms that regulate gene expression in myeloid cells are poorly understood at present. The promoter of CD18, the β chain of the leukocyte integrins, directs myeloid expression of the linked luciferase reporter gene. Deletion analysis indicates that a 79-nt fragment of the CD18 promoter, which contains two PU.1 transcription factor-binding sites, directs expression in myeloid cells. Mutagenesis of these sites significantly decreases the myeloid activity of the CD18 promoter and causes a corresponding decrease in PU.1 binding. Thus, the PU.1 transcription factor is required for myeloid activity of the CD18 promoter.

A PU.1-binding site consensus sequence (GAGGAA) has been defined based on binding sites in the simian virus 40 enhancer (2) and murine β globin intervening sequence 2 (14). This consensus sequence is present in several leukocyte-specific promoters (13, 15–17), including CD18 (7, 18). Because PU.1 is expressed by B lymphocytes and myeloid cells (2, 19), it is a candidate for a transcription factor that regulates expression of these genes. PU.1 is now shown to be critical for the activity of CD18, as well as for CD11b, a leukocyte integrin α chain that forms a heterodimer with CD18. Interestingly, in the CD11b promoter, PU.1 binds to a site that does not conform to this consensus sequence, yet a nearby GAGGAA sequence is not bound by PU.1 (3, 4). Thus, the functional role of the potential PU.1 sites in other leukocyte-specific genes must be individually defined.

Both CD18 and CD11b are transcriptionally regulated during myeloid differentiation (7, 8), and both require PU.1 binding for myeloid activity. B lymphocytes express both PU.1 and CD18, but they do not express CD11b. This result suggests that additional transcription factors or modifications of PU.1 are required to permit B-lymphocyte expression of CD18. Alternatively, other factors may specifically repress CD11b expression in B lymphocytes. Further analysis may reveal DNA sequences and trans-acting factors that account for the different tissue-specific expression of these two genes.

Recently, Böttinger *et al.* (20) also identified this region of the CD18 promoter as being required for CD18 promoter activity. They suggested a role for *ets* family members but failed to identify PU.1-binding activity to the CD18 promoter. We present direct evidence of the role of PU.1 in binding to these sites. PU.1 contains a PEST domain that renders it susceptible to protease cleavage (2). We have observed that PU.1 is labile; insufficient protease inhibition causes loss of detectable PU.1-binding activity. The failure of Böttinger *et al.* to identify PU.1 binding to the CD18 promoter may be due to proteolytic degradation of PU.1 in their nuclear extracts.

PU.1, like other *ets* factors (21, 22), interacts with heterologous factors to activate transcription. PU.1 binds to a site in the immunoglobulin κ 3′ enhancer. However, high-level expression is seen only when PU.1 recruits NF-EM5, a second B-cell-restricted transcription factor that binds only in the presence of PU.1 (23). The interaction between PU.1 and NF-EM5 requires phosphorylation of a specific serine residue in PU.1 (24). Myeloid nuclear proteins form DNase I footprints on the CD18 promoter that extend beyond the core GAGGAA PU.1-binding sites. These extended footprints may represent contact of flanking nucleotides by PU.1 itself or binding to the CD18 promoter of another, potentially cooperating transcription factor.

PMA treatment of U-937 cells induces greater monocytic differentiation, increases CD18 transcription, and activates the transfected CD18 promoter. However, we have shown that PMA treatment of U-937 cells does not substantially alter PU.1 binding to the CD18 promoter. Although PU.1 is re-

quired for myeloid activity of the CD18 promoter, it appears that either posttranslational modifications of PU.1 or additional factors are required for increased CD18 expression during myeloid differentiation.

We have shown that myeloid expression of CD18 depends on the integrity of the PU.1-binding sites in its promoter. Mutagenesis indicates that both sites are required for maximal promoter activity. PU.1 binding to these sites may entail cooperativity, either involving PU.1 homodimers or higher order complexes with other proteins. Whether a posttranslational modification of PU.1 or recruitment of a second transcription factor is required for the increase in CD18 expression during myeloid differentiation remains to be elucidated. To date, a myeloid-specific partner that interacts with PU.1 to regulate transcription has not been identified. We speculate that such a factor may act with PU.1 to govern the increase in CD18 transcription that occurs during myeloid differentiation.

Note Added in Proof. The crucial role of PU.1 in hematopoiesis was recently indicated by the ability of PU.1-binding oligonucleotides to disrupt granulocte/macrophage colony formation from bone marrow progenitors (25). Furthermore, targeted disruption of PU.1 in mice results in an embryonic lethal phenotype with a multilineage hematopoietic defect, including a loss of myeloid and lymphoid colony formation (26).

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1. Mitchell, P. J. & Tjian, R. (1989) *Science* **245,** 371–378.
2. Klemsz, M. J., McKercher, S. R., Celada, A., Van Beveren, C. & Maki, R. A. (1990) *Cell* **61,** 113–124.
3. Pahl, H. L., Scheibe, R. J., Zhang, D. E., Chen, H. M., Galson, D. L., Maki, R. A. & Tenen, D. G. (1993) *J. Biol. Chem.* **268,** 5014–5020.
4. Chen, H. M., Pahl, H. L., Scheibe, R. J., Zhang, D. E. & Tenen, D. G. (1993) *J. Biol. Chem.* **268,** 8230–8239.
5. Todd, R. F. & Freyer, D. R. (1988) *Hematol. Oncol. Clin. North Am.* **2,** 13–31.
6. Rosmarin, A. G., Weil, S. C., Rosner, G. L., Griffin, J. D., Arnaout, M. A. & Tenen, D. G. (1989) *Blood* **73,** 131–136.
7. Rosmarin, A. G., Levy, R. & Tenen, D. G. (1992) *Blood* **79,** 2598–2604.
8. Pahl, H. L., Rosmarin, A. G. & Tenen, D. G. (1992) *Blood* **79,** 865–870.
9. Henikoff, S. (1984) *Gene* **28,** 351–359.
10. Nordeen, S. K. (1988) *BioTechniques* **6,** 454–458.
11. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11,** 1475–1489.
12. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K., eds. (1990) *Current Protocols in Molecular Biology* (Greene and Wiley, New York).
13. Zhang, D.-E., Hetherington, C., Chen, H.-M. & Tenen, D. G. (1994) *Mol. Cell. Biol.* **14,** 373–381.
14. Galson, D. L. & Housman, D. E. (1988) *Mol. Cell. Biol.* **8,** 381–392.
15. Hohn, P. A., Popescu, N. C., Hanson, R. D., Salvesen, G. & Ley, T. J. (1989) *J. Biol. Chem.* **264,** 13412–13419.
16. Roberts, W. M., Shapiro, L. H., Ashmun, R. A. & Look, A. T. (1992) *Blood* **79,** 586–593.
17. Han, J., Unlap, T., & Rado, T. A. (1991) *Biochem. Biophys. Res. Commun.* **181,** 1462–1468.
18. Agura, E. D., Howard, M. & Collins, J. (1992) *Blood* **79,** 602–609.
19. Ray, D., Bosselut, R., Ghysdael, J., Mattei, M. G., Tavitian, A. & Moreau-Gachelin, F. (1992) *Mol. Cell. Biol.* **12,** 4297–4304.
20. Böttinger, E. P., Shelley, C. S., Farokhzad, O. C. & Arnaout, M. A. (1994) *Mol. Cell. Biol.* **14,** 2604–2615.
21. Brown, T. A. & McKnight, S. L. (1992) *Genes Dev.* **6,** 2502–2512.
22. Dalton, S. & Treisman, R. (1992) *Cell* **68,** 597–612.
23. Pongubala, J. M., Nagulapalli, S., Klemsz, M. J., McKercher, S. R., Maki, R. A. & Atchison, M. L. (1992) *Mol. Cell. Biol.* **12,** 368–378.
24. Pongubala, J. M. R., Van Beveren, C., Nagulapalli, S., Klemsz, M. J., McKercher, S. R., Maki, R. A. & Atchison, M. L. (1993) *Science* **259,** 1622–1625.
25. Voso, M. T., Burn, T. C., Wulf, G., Lim, B., Leone, G. & Tenen, D. G. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 7932–7936.
26. Scott, E. W., Simon, M. C., Anastasi, J. & Singh, H. (1994) *Science* **265,** 1573–1577.