The Human $\beta 2$ Integrin CD18 Promoter Consists of Two Inverted Ets *cis* Elements

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To define the minimal promoter responsible for expression of CD18 in myeloid and lymphoid cells, we generated 5' and 3' deletion constructs of a segment extending 785 bp upstream and 19 bp downstream of a major transcription start site and determined their effects on driving expression of the luciferase reporter gene in transfected hematopoietic cell lines. A region extending from nucleotides (nt) –302 to +19 was sufficient for cell-restricted and phorbol ester-inducible expression. DNase I footprinting of this region revealed two adjacent protected segments extending from nt –81 to –68 (box A) and –55 to –41 (box B). When a construct of 47 nt in length containing box A and box B and lacking other 3' or 5' elements was cloned into a promoterless vector, it conferred tissue-specific and phorbol ester-inducible expression. Gel retardation revealed that the protein components of two major protein-DNA complexes that form on both box A and box B and are required for transcriptional activation are members of the Ets oncoprotein family; one is related to the GA-binding protein (GABP), and the other is related to PU.1/Spi-1. The minimal CD18 promoter, lacking TATA, CAAT, and initiator elements and consisting primarily of *Ets* repeats, may exemplify an emerging class of promoters with which the concerted binding of *Ets* factors is necessary and sufficient to mediate transcriptional activation through direct recruitment of the basal transcription machinery.

The CD11/CD18 ($\beta 2$ integrin) family consists of three heterodimeric cell surface receptors, CD11a/CD18 (LFA-1 and TA-1), CD11b/CD18 (CR3, Mo1, and Mac-1), and CD11c/CD18 (p150,95). Unlike that of many other integrins, $\beta 2$ integrin expression is restricted to leukocytes. CD11a and CD18 are expressed on virtually all mature leukocytes. Expression of CD11b and CD11c is restricted to phagocytes (polymorphonuclear cells and monocytes/macrophages) and large granular lymphocytes. $\beta 2$ integrins play major roles in the immune system (reviewed in reference 3). They are critical for the recruitment of circulating leukocytes to inflammatory sites, in that they are responsible for the establishment of firm adhesion and transendothelial migration. Each receptor also has additional distinct functional profiles. CD11a/CD18 plays an important role in lymphocyte proliferation, cell-mediated cytotoxicity, monocyte-lymphocyte and B-T-cell adhesion, and T-helper activity. CD11b/CD18 is an oligospecific receptor that is primarily involved in neutrophil adhesion-dependent functions, such as chemotaxis, phagocytosis, homotypic and heterotypic adhesion, and antibody-dependent cytotoxicity. It shares many of these functions with CD11c/CD18. $\beta 2$ integrins also mediate the cytopathic effects induced by human immunodeficiency virus type 1 (HIV-1), as HIV-1-induced monocyte aggregation, a critical step in viral dissemination, is prevented by anti-CD18 monoclonal antibodies (reviewed in reference 41).

The critical biologic role of $\beta 2$ integrins in the immune system was established by the discovery that their inherited deficiency (leukocyte adhesion molecule [Leu-CAM] deficiency) in several mammalian species, including humans, leads to recurrent and often fatal pyogenic infections due to the inability of the $\beta 2$ integrin-deficient phagocytes to migrate into

infected tissues and to ingest invading microorganisms. Leu-CAM deficiency results from inherited or spontaneous mutations in CD18, impairing its expression and association with the normally expressed CD11 subunits (reviewed in reference 2). Elucidation of the critical role of $\beta 2$ integrins in leukocyte extravasation suggested novel therapeutic approaches to the treatment of a large number of noninfectious but equally life-threatening diseases resulting from uncontrolled phagocyte extravasation. Such diseases include ischemia-reperfusion syndromes (as occur in myocardial infarction, hemorrhagic shock, burns, frostbite, cerebral edema, reattachment of a severed limb, or a kidney graft), autoimmune disorders (including diabetes mellitus and arthritis), and allograft rejection (reviewed in reference 2).

Inflammatory mediators exert important controls on the levels of expression of $\beta 2$ integrins in leukocytes. Some of these controls are exerted at the transcriptional level, leading to activation (56) of one or more CD11 genes (located on chromosome 16p11-13.1 [4, 14]) and of the CD18 gene (located on chromosome 21q22.3 [9]). The synthesized subunits assemble noncovalently in a 1:1 stoichiometry, a step that is followed by carbohydrate processing and transport to the cell surface. Disruption of this association in the endoplasmic reticulum, as occurs in congenital Leu-CAM deficiency, abolishes surface expression. The underlying basis for the coordinate transcriptional upregulation of these integrin subunits during inflammatory reactions is unknown. A detailed understanding of the underlying factors is an essential component in development of specific agents, such as peptide nucleic acids (22) or transcription factor antagonists (28), that can down-regulate expression of the CD11/CD18 genes in such common pathologic conditions as autoimmune disorders and allograft rejection.

The CD11/CD18 genes also encode differentiation antigens whose expression is tightly regulated during hematopoiesis and is affected during leukemogenesis. The chromosomal segments housing the CD11 and CD18 genes are involved in rearrange-

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ments found in patients with various forms of blood malignancies, including acute myelomonocytic leukemia [with inv (16)(p13;q22) or t(16;16)(p13;q22) in chromosome 16 (24, 30)] and the blast phase of chronic myelogenous leukemia [with t(3;21)(q26;q22) (57)]. The region housing the CD18 gene also contains the proto-oncogenes *ets-1* and *ets-2*. Microduplications of this region occur in Down's syndrome and may contribute to the increased incidence of leukemia with this disease (59). Although no direct evidence links altered CD11/CD18 expression to leukemogenesis per se, a large body of data suggests that cell-cell and cell-matrix interactions (some mediated by $\beta 2$ integrins) are important in regulating differentiation and maturation of hematopoietic cells (52).

The regulated expression of the CD11 and CD18 genes has been studied in established hematopoietic lines and found to be a highly specific marker for lineage- and stage-specific differentiation of leukocyte subpopulations (35, 54). For example, induction of differentiation of the promyelocyte cell line HL60 (along the monocytic or granulocytic lineages) or of the monoblastic cell line U937 (into the monocytic cell lineage) with phorbol esters or retinoic acid leads to transcriptional induction of mRNAs for both the CD11 and the CD18 genes and to expression of the CD11/CD18 heterodimers on the cell surface (23, 56). These studies suggest that although they are distinct, these genes may share common control elements. To define these elements, analysis of the promoter regions of the CD11 genes (12, 15, 33, 39, 40, 61, 62) and the CD18 gene (1, 55) has recently begun. However, the specific control elements regulating the CD18 gene remain undefined.

In this report we describe the identification and characterization of *cis*-acting elements that define the CD18 promoter. We found that the CD18 promoter consists of two closely spaced and inverted short elements, boxes A and B, in proximity to the multiple transcription start sites. The two *cis*-elements are necessary and sufficient for tissue-specific expression of the CD18 gene and contribute to its upregulation during phorbol ester-induced cellular differentiation or activation. The two elements were protected in a tissue-specific manner *in vitro*, and each interacted with two major factors, related to the GA-binding protein (GABP) and PU.1/Spi-1 members of the Ets family of oncoproteins. Similar factors were also found to interact with the CD11a promoter. This is the first report of a role of a GABP-related factor in gene expression in hematopoietic cells and suggests that concordant binding of Ets factors through repeated elements may be a general feature of some TATA-less promoters that direct tissue- and stage-specific gene expression through clustered and multiple transcription start sites.

MATERIALS AND METHODS

Isolation of the 5'-flanking region of the CD18 gene. A DNA fragment encompassing the 5'-flanking region between nucleotides (nt) -785 and +19 relative to the most 3' major transcription initiation site was amplified from total genomic DNA by PCR with two primers designed according to the CD18 genomic DNA sequence (forward primer, 5'-GGG TAGTAATGTTTCTGAGTC, nt -850 to -830; reverse primer, 5'-CTTTGCTACCACTCTGCCC, nt -70 to -52 [1]). The blunt-ended fragment was then ligated into the *Sma*I site of the expression vector pATLuc (62) to generate the plasmid construct -785/+19.

DNA sequencing. Double-stranded DNA was sequenced by the dideoxynucleotide chain termination-extension method with Sequenase (United States Biochemical, Cleveland, Ohio) (60).

Cell culture. The T-cell lines Jurkat and CEM, the B-cell line IM9, the monoblastic cell line U937, and the epithelial cell line HeLa were obtained from the American Type Culture Collection and grown according to their specifications. Phorbol 12-myristate 13-acetate (PMA; Sigma) was used at a concentration of 100 ng/ml where indicated.

Plasmid construction. The activity of the CD18 promoter was assessed by using the expression vector pATLuc, which contains a promoterless firefly luciferase reporter gene (62). The PCR and restriction endonuclease digestion were used to generate the fragments from nt -532 to +19, -302 to +19, -79 to +19, and -66 to +19 relative to the most 3' major transcription initiation site (55). These fragments were subcloned into the *Sma*I site of pATLuc to generate, respectively, the -532/+19, -302/+19, -79/+19, and -66/+19 constructs. The constructs -785/-56 and -81/-35 were produced by subcloning the blunt-ended PCR-generated CD18 gene fragments into the filled-in *Sal*I site and the *Sma*I site of pATLuc, respectively. Point mutations were introduced within the -81/-35 construct as described previously (37).

Transfection. U937, Jurkat, CEM, and IM9 cells (4×10^7) were transfected by electroporation (44, 61) with 45 μ g of a luciferase test plasmid together with 15 μ g of plasmid pSV- β -gal (Promega Corp., Madison, Wis.) containing the *lacZ* gene. After electroporation, cells were split equally and grown in the absence or presence of PMA for 16 h at 37°C before processing. Afterwards, cells were processed for assays of β -galactosidase and luciferase activity (61). HeLa cells were transfected by the calcium phosphate precipitation method (20). Adherent HeLa cells in Falcon tissue culture dishes (150 by 25 mm) were exposed to the identical amounts of plasmid DNAs used in the electroporation procedure, in 20 ml of media for 7 h at 37°C, and then to a glycerol shock. The cells were then incubated in the absence or presence of PMA for 16 h before being processed as described above. Each transfection was performed in parallel with a transfection of the negative control plasmid, pATLuc.

Nuclear extracts. Nuclear extracts were prepared according to the method described by Dignam et al. (17), with modifications consisting of overnight dialysis at 4°C and the addition of the protease inhibitors pepstatin, leupeptin, and aprotinin to the nuclear extract.

DNase I protection analysis. DNA fragments containing the region between nt -302 and +19 were generated by PCR with one 5'-end-labeled oligonucleotide primer (labeled by using T4 polynucleotide kinase and [γ -³²P]ATP) and one unlabeled oligonucleotide primer. Radiolabeled DNA fragments (10,000 cpm) were incubated on ice for 15 min with 7.5 μ g of nuclear extract by using the Stratagene HotFoot buffer system [0.1 M KCl, 1 mM dithiothreitol, 20% glycerol, 12.5 mM MgCl₂, 10 μ M ZnSO₄, 0.1% Nonidet P-40, 25 mM Tris (pH 7.5), 1 μ g of poly(dI-dC) · poly(dI-dC)] and then digested with 0.0125 to 0.125 U of DNase I for 2 min at room temperature. Phenol-chloroform extractions and ethanol precipitations were performed prior to denaturing polyacrylamide gel electrophoresis and autoradiography.

Electrophoretic mobility shift assays. DNA probes were generated by annealing complementary oligonucleotides which had been radiolabeled at their 5' ends by using T4 polynucleotide kinase and [γ -³²P]ATP. Nuclear extract (1 to 3 μ g) was incubated at 4°C for 15 min in 70 mM KCl-5 mM NaCl-20 mM Tris-Cl (pH 7.5)-0.25 mM MgCl₂-0.5 mM EDTA-1 mM dithiothreitol-10% glycerol-2.4 μ g of poly(dI-dC) · poly(dI-dC) with a 150 to 500 molar excess of unlabeled competitor probe where indicated. Radiolabeled probe (10,000 cpm, 0.05 to 0.15 ng) was then added, and the incubation was continued

for 30 min prior to native gel electrophoresis and autoradiography. Band intensity was quantified with an LKB densitometer.

RESULTS

A 302-bp fragment from the 5' region of the CD18 gene mediates tissue-specific and phorbol ester-inducible gene expression. Previous studies have demonstrated that CD18 gene expression is regulated at the transcriptional level in normal as well as certain leukemic cell lines, such as the monoblastic U937 or the T-lymphoblastoid cell line Jurkat (1, 23, 39). Three 5'-deleted CD18 promoter-luciferase constructs, $-785/+19$, $-532/+19$, and $-302/+19$, were transfected into these cell lines to identify the functional *cis*-elements. The relative transcriptional activity conferred by each construct was expressed as the fold activity above the background luciferase activity produced by the promoterless pATLuc vector, corrected for transfection efficiency. All three constructs resulted in baseline transcriptional activities in U937 and Jurkat cells. Baseline levels were 150- to 300-fold higher than those seen with pATLuc alone and 5- to 10-fold higher than that seen in the CD18-negative epithelial cell line HeLa (Fig. 1). Treatment of cells with PMA at concentrations previously shown to induce transcriptional upregulation of the CD18 gene (100 ng/ml for 16 h at 37°C) led to a significant 2.9-fold ($-302/+19$) to 4-fold ($-785/+19$) increase of transcriptional activity in U937 and a significant 1.6-fold increase (for $-785/+19$ and $-302/+19$) in Jurkat cells (Fig. 1). Similar results were seen with the CEM T-cell line (data not shown). In HeLa cells, PMA treatment had no significant effect. These results suggested that the promoter region, encompassing nt -302 to $+19$, is sufficient to direct tissue-specific expression of the CD18 gene and contains phorbol ester-responsive elements required for transcriptional induction of the CD18 gene during differentiation of monoblastic or T-cell lines. In U937 (but not in Jurkat) cells, a significant increase in baseline transcriptional activity occurred when the region between nt -532 and -303 was deleted (Fig. 1), suggesting the presence of a myeloid-specific suppresser element in this region.

The 302-bp CD18 fragment contains two major binding sites for nuclear factors. The interaction of nuclear proteins with the $-302/+19$ region of the CD18 promoter was examined by DNase I footprinting. 5'-end-labeled PCR fragments (10,000 cpm) were incubated with nuclear extracts prepared from uninduced and PMA-induced U937, the T-cell line CEM, and HeLa cells. The reaction mixture was then digested with DNase I, phenol-chloroform extracted, and ethanol precipitated, and the DNA was analyzed by denaturing polyacrylamide gel electrophoresis. The G/A ladder of the respective radiolabeled DNA fragments generated by Maxam-Gilbert reaction served as a marker. This analysis revealed that two regions, referred to as box A (nt -81 to -68) and box B (nt -55 to -41), were protected in both the coding and noncoding strands (Fig. 2A) in the monoblastic cell line U937 (before and after induction with PMA) as well as in CEM cells. Two hypersensitive sites were seen, respectively, in the middle of box B in the antisense strand (Fig. 2A, open circle) and the 3' end of box A in the sense strand (Fig. 2A, asterisk). No protection in either strand was seen in the nonleukocytic HeLa cell line. However, three hypersensitive sites (two in the antisense strand [Fig. 2A, closed circles] and one in the sense strand [Fig. 2A, double asterisk]) were located either at the boundaries or outside box A and box B. Inspection of boxes A and B (Fig. 2B) revealed an identity of 8 of 9 and 9 of 9 bp, respectively, with a known binding site (5' CAGGAAGTG-3',

FIG. 1. Deletion analysis of the CD18 promoter. Luciferase reporter gene activity, corrected for transfection efficiency, is expressed as fold activity above the background activity conferred by the promoterless control plasmid pATLuc in untreated (–) and PMA-treated (+) cells. Black histograms and shaded histograms demonstrate, respectively, the activities in untreated (–) or PMA-treated (+) U937, Jurkat, and HeLa cells. Each histogram represents the mean $\pm$ standard deviation (error bar) of three to five transfection experiments. The portions of the CD18 gene and cell lines used in transfection assays are shown at the bottom. ** depicts statistical significance ($P < 0.01$) (see the text for details).

designated the PEA3 motif) for the DNA-binding proteins Ets-1 and Ets-2 present in the polyomavirus enhancer (68). Immediately upstream of the putative PEA3 motif, box B also contained the sequence 5'-GAGGAA-3', a potential binding site for the Ets proteins PU.1/Spi-1 (19, 26, 42) and Spi-B (50) (Fig. 2B).

A 47-bp fragment of the CD18 gene containing box A and box B confers cell-specific and phorbol ester-inducible expression of the CD18 gene. To evaluate the functional importance of box A and box B in myeloid and lymphoid cells, we prepared a series of additional constructs (Fig. 3), using the pATLuc vector as described above. These constructs were transfected in parallel into leukocytic and epithelial cell lines, and their effects on expression of the luciferase gene were compared with that of the $-302/+19$ construct. None of the 5' and 3' deletion constructs were expressed by the CD18-negative cell lines IM9 and HeLa (Fig. 3). In U937 cells, the $-79\{A \mid B\}+19$ construct ($\{A \mid B\}$ indicates that boxes A and B are present) maintained the same baseline expression seen for the $-302/+19$ construct. It also retained a significant portion of the phorbol ester response. The magnitude of this

FIG. 2. DNase I protection analysis of the proximal CD18 promoter identifies two footprints, box A and box B. (A) A radiolabeled PCR fragment, encompassing the CD18 gene from nucleotides -302 to +19, was incubated with 7.5 μ g of nuclear extract derived from U937, PMA-treated U937, CEM (a T-cell line), and HeLa cells (lanes 3 to 6, respectively). Following digestion with DNase I, samples were run on denaturing polyacrylamide gels in parallel with a G+A sequence ladder generated with the same fragment (lane 1 [M]), as well as a fragment digested with DNase I in the absence of nuclear extract (lane 2 [DNA]). The results of experiments with the sense and the antisense strands are shown. Footprinted regions are designated A and B, and the protected sequences are shown in the lateral extensions. Circles and asterisks mark DNase I hypersensitive sites in the antisense and sense strands, respectively. Open and closed circles in the antisense strand (and single and double asterisks in the sense strand) mark hypersensitive sites observed in leukocytic and nonleukocytic cell lines, respectively. The dashed lines indicate uncertain boundaries of protection. Open arrows denote the positions and orientations of the conserved 5'-AGGAAGTG-3' sequence elements. (B) The CD18 gene sequence between nt -302 and +19 relative to the most 3' major transcription initiation site (indicated as +1). The sequence matched exactly with that reported by Rosmarin et al. (55). Two nucleotides within box A (T and C at positions -75 and -73, respectively) are missing from the sequence reported by Agura et al. (1). The footprinted regions labeled "BOX A" and "BOX B" are lightly shaded. Conserved tandem Ets motifs are boxed. The positions of the hypersensitive sites indicated in Fig. 2A are also shown. Potential binding sites for the nuclear proteins CREB, AP-1, and NF-IL-6 (nuclear factor for interleukin-6 expression) (72) are underlined. Thick and thin arrows indicate the previously identified major and minor transcription start sites (1, 55).

response, however, was 37% lower ($P < 0.05$) than that obtained with the $-302/+19$ construct, suggesting that *cis*-acting elements between nucleotides -302 and -80 are required to achieve an optimal response to phorbol ester. The region between -302 and -80 contains a potential AP-1 site (58), a CREB site (53), and two potential NF-IL6 motifs (72) that may contribute to PMA-induced activation. Deletion of the 3' region between nt -34 and $+19$ ($-81\{A \mid B\}-35$) did not affect baseline expression or PMA induction compared with that of the $-79\{A \mid B\}+19$ construct (Fig. 3). These data limit the promoter region of CD18 to a 47-bp segment (nt -81 and -35) containing box A and box B. Specific deletions of either box A ($-66\{-/B\}+19$) or box B ($-785\{A \mid -\}-55$) dramatically reduced luciferase activity in U937 cells (Fig. 3).

The $-79\{A \mid B\}+19$ construct also directed expression of the luciferase gene (Fig. 3) in lymphoid cells. Expression in Jurkat (Fig. 3) and CEM (data not shown) cells also required the presence of box A and box B as deletion of either element abolished expression. The magnitude of baseline and inducible luciferase expression directed by the $-79\{A \mid B\}+19$ construct in Jurkat cells was significantly lower than that produced by the $-302\{A \mid B\}+19$ construct (Fig. 3). Interestingly, removal of the region between nt -34 and $+19$ in the $-81\{A \mid B\}-35$ construct reversed the reduction in baseline expression of the $-79\{A \mid B\}+19$ construct and somewhat enhanced the phorbol ester response (Fig. 3). Taken together, these results suggest that the CD18 promoter region encompassing both box A and box B is necessary and sufficient to direct tissue-specific and phorbol ester-inducible gene expression in lymphoid cells.

Nuclear factors binding to box A and box B. To identify DNA-binding proteins that interact with box A and box B, we performed electrophoretic mobility shift assays. Incubation of oligonucleotide probes corresponding to box A or box B with nuclear extracts derived from uninduced or PMA-induced U937, Jurkat, CEM, IM9, and HeLa cells, resulted in the formation of several DNA-protein complexes (Fig. 4). When box A was used as a probe, a major complex, designated BA-1, was observed (Fig. 4A). This complex increased in intensity after phorbol ester-induced differentiation of U937 cells (Fig. 4A, compare lanes 2 and 4). In Jurkat cells (Fig. 4A, lanes 13 and 15), the increase in intensity was less dramatic (~1.5-fold). A second complex with a higher mobility, designated BA-1 α , was observed in U937 cells but not in the other cell lines and did not increase in intensity after PMA induction. A major MS-1 complex and a minor MS-3 complex were seen only in U937 cells (Fig. 4A, lanes 2 and 4). A fifth complex, MS-2R, was seen in the CD18-negative cell lines IM9 and HeLa (Fig. 4A, lanes 8 and 10). Formation of all five complexes was diminished by the unlabeled box A oligonucleotide.

BA-1, BA-1 α , MS-1, and MS-3, but not MS-2R, were also detected when nuclear extracts from U937 cells were incubated with the radiolabeled box B element (Fig. 4B). This conclusion was based on the apparent identical mobility of the complexes (compare Fig. 4A and B) as well as on the ability of box B to compete with box A for the binding of BA-1, BA-1 α , MS-1, and MS-3 (Fig. 5A, lane 19). Box B did not compete with box A for binding to MS-2R (Fig. 5B, lane 9), confirming the inability of the box B oligonucleotide to form this complex. In nuclear extracts derived from CEM, IM9, HeLa, and Jurkat cells, box B bound BA-1 as well as a second complex (MS-4) that has a mobility similar to that of MS-3 but appears distinct on the basis of its different cell distribution pattern (Fig. 4B) as well as the lack of competition by a 500-molar excess of the unlabeled box A oligonucleotide (data not shown). No change

FIG. 3. Effect of deletions involving box A or box B on cell-specific and inducible gene expression. Results of transfection assays are displayed as described in the legend to Fig. 1. The transfection data for the $-302\{A|B\}+19$ construct are the same as in Fig. 1 and shown here for comparison. The transfected portions of the CD18 gene and cell lines used are indicated at the bottom. Each histogram represents the mean $\pm$ standard deviation (error bar) of three to five experiments. *, $P < 0.05$; **, $P < 0.01$ (comparing expression of the respective construct in the absence and presence of PMA).

in the appearance of MS-4 was observed after PMA induction (Fig. 4B, lanes 13 and 15).

Competition assays using oligonucleotides containing consensus-binding sequences of known transcription factors were next performed to evaluate the nature of the identified DNA-binding proteins. Binding of the radiolabeled box A to BA-1, BA-1 α , MS-1 and MS-3 was not diminished by oligonucleotides containing AP-1-, AP-2-, AP-3-, NF- κ B-, OCT-1-, CREB-, GRE-, SP1-, NF-1-, or GATA-1-binding sequences (each used at 500-fold molar excess [Fig. 5A, lanes 4 to 13]). However, a simian virus 40 DNA fragment containing the recognition site of the Ets protein PU.1/Spi-1 (26, 50) competed partially for BA-1 and BA-1 α (Fig. 5A, lane 14) and completely for MS-1 and MS-3. An identical result was observed when a shorter version of the simian virus 40 oligonucleotide containing the core PU.1 recognition site and its flanking regions (CCTCTGAAAGAGGAAGCTTGGT) was used (data not shown). These data suggest that MS-1 and MS-3 may be related to each other and to PU.1/Spi-1. Interestingly, two previously characterized CD11a promoter elements, GAGA (62) and LEU, competed for the binding of one or more of the factors forming over the CD18 minimal promoter

(Fig. 5A, lanes 15 and 17). The CD11a GAGA element has been previously shown to interact with at least two putative factors, MS-1, a PU.1-like factor, and MS-2, a factor defined by its binding to the core nucleotide sequence (CCT/ACCC, the MS-2 element) (62). The CD11a LEU box contains a putative Ets-binding site. The GAGA element competed effectively for the binding of BA-1, BA-1 α , MS-1, and MS-3 complexes (Fig. 5A, lane 15), suggesting that the GAGA element also binds to BA-1 and BA-1 α . The LEU element, which contains a putative Ets-binding site, competed effectively with box A for the binding to BA-1 and BA-1 α but only partially for binding to MS-1 (Fig. 5A, lane 17). Two previously defined elements in the CD11a promoter, the LYM box and the MS-2 element (62), did not compete for BA-1, BA-1 α , MS-1 and MS-3 (Fig. 5, lanes 16 and 18), supporting previous conclusions that the complexes forming over these elements are not related to the Ets family (62).

The MS-2R complex that forms over box A was not competed for by any of the consensus sequences described above, with the exception of the CD11a MS-2 element (Fig. 5B, lane 6), suggesting that MS-2R is related to MS-2. However, minimal competition was observed when the GAGA element,

FIG. 4. Electrophoretic mobility shift assays (EMSA) showing the protein-DNA complexes that form on boxes A and B. Radiolabeled oligonucleotides (5'-CACCACCTTCCTCCAAGGAG, representing box A [A] and 5'-CTGAGAGGAACAGGAAGTGTCAGGA, representing box B [B]) were incubated with no nuclear extract (lanes 1 and 12) or nuclear extracts (1.3 μ g) prepared from U937 promonoblastic cells (lanes 2 and 3), U937 cells induced to differentiate along the monocytic pathway with PMA (lanes 4 and 5), the T-lymphoblastoid cell line CEM (lanes 6 and 7), IM9 (a B-lymphoblastoid cell line) (lanes 8 and 9), HeLa epithelial cells (lanes 10 and 11), and the T-cell line Jurkat in the absence (-PMA) or presence (+PMA) of PMA induction (lanes 13 and 14 and 15 and 16, respectively). Binding reactions were performed either in the absence (-) of unlabeled specific competitor DNA or in the presence (+) of a 500 molar excess of the respective unlabeled oligonucleotide as indicated. The DNA-binding activities of BA-1, BA-1 α , MS-1, MS-2R, MS-3, and MS-4 are indicated with arrows. Nonspecific DNA-binding activities are indicated by open arrowheads.

another MS-2-binding motif, was used (Fig. 5B, lane 5), suggesting that the two complexes may not represent a single factor.

The BA-1 complex is homologous to GABP. The competition studies strongly suggested that BA-1, BA-1 α , MS-1, and MS-3 are members of the Ets family of transcription factors. In order to determine whether these complexes represent known members of this family, supershift analyses were carried out using available antibodies to three known members of this family, GABP α , PU.1, and Ets-1, and to GABP β , a notch-related non-Ets protein that forms a heteromeric complex with GABP α , thereby enhancing its DNA-binding affinity (64). Antiserum specific to GABP α abolished binding of BA-1, as well as BA-1 α , to the box A probe (Fig. 6, lane 3). On the other

FIG. 5. Competition analysis of BA-1, BA-1 α , MS-1, MS-3, and MS-2R-binding activities with oligonucleotides to known transcription factors. (A) Radiolabeled box A oligonucleotide was incubated with no nuclear extract (lane 1) or nuclear extract (1.3 μ g) from PMA-treated U937 cells in the absence of unlabeled specific competitor DNA (lane 2) or the presence of a 500 molar excess of the following unlabeled competitor DNA fragments: box A (WT, wild type) (lane 3); DNA fragments representing, respectively, recognition sites of the transcription factors AP-1, AP-2, AP-3, NF- κ B, OCT-1, CREB, GRE, SP-1, NF-1, and GATA-1 (reference 62 and Stratagene Cloning Systems, La Jolla, Calif.) (lanes 4 to 13, respectively); the simian virus 40 DNA fragment 5'-GATCTGAAATAACCTCTGAAAGAGGAACCTGGTTAGGTA, representing the recognition site for the murine Ets transcription factor PU.1 (26) (lane 14); DNA fragments of the CD11a promoter (62) spanning positions -89 to -70 (5'-TTACACTTCCTCCTGAACC, GAGA), -45 to -25 (5'-GCACACCTCCCTCCCCGCCTG, MS-2), -115 to -95 (5'-GCAGGCAC AGGAAATGTCTCTA, LEU), and -140 to -116 (5'-TTTGGATGATGTGAAAA TGC AAG, LYM), respectively (lanes 15 to 18); and an oligonucleotide representing box B (lane 19). DNA-binding activities of BA-1, BA-1 α , and MS-1, as well as MS-3, are marked with arrows. (B) An experiment equivalent to that described for panel A, but performed with nuclear extract (1.3 μ g) from IM9 cells to evaluate the effect of various oligonucleotides to compete for the MS-2R complex.

hand, antiserum specific for GABP β did not affect BA-1 α binding but abolished the BA-1 complex (Fig. 6, lane 4). Similar results were seen when box B was used as a probe (data not shown), providing further evidence that the same BA-1 and BA-1 α complexes interact with the box A and box B elements. These results suggest that the higher-mobility complex, BA-1 α , may represent DNA-binding activity related to that of the α

FIG. 6. Effect of antibodies to known Ets members on BA-1 and MS-1 complexes. Supershifts were performed as described in Materials and Methods with the radiolabeled box A oligonucleotide probe, which was added to no nuclear extract (lane 1), nuclear extract from PMA-treated U937 cells (lane 2), or nuclear extract from PMA-treated U937 cells preincubated for 15 min at 4°C with 1 μ l of rabbit polyclonal antisera against GABP α (lane 3), GABP β (lane 4), PU.1 (lane 5), Ets-1 (lane 6), or rabbit preimmune serum (pre) (lane 7). DNA-binding activities of BA-1, BA-1 α , and MS-1 are indicated by arrows. Antisera were raised against a peptide (residues 362 to 374) of human Ets-1 (Santa Cruz Biotechnology, Inc.), against a peptide (residues 33 to 45) of mouse PU.1 (a gift of Richard Maki), and against the whole recombinant rat proteins GABP α and GABP β , (a gift of Thomas Brown).

subunit of GABP and that BA-1 may be related to a heteromeric GABP complex consisting of α and β subunits. The antisera against PU.1 and Ets-1, as well as a rabbit preimmune serum, did not affect the formation or mobility of the BA-1 and BA-1 α complexes. All antisera also failed to recognize the MS-1 complex (Fig. 6).

Mutational analysis of the nuclear factor-binding sites. To identify sites that are vital for protein-DNA interactions, we assayed MS-1, BA-1, and MS-2R binding to box A with oligonucleotides containing selected mutations (Fig. 7C). The mutations involved residues that were previously shown to be important in DNA binding of members of the Ets family (8, 38, 66) (in the case of BA-1 and MS-1) or the related CD11a MS-2 (in the case of MS-2R) (62). The M1 mutation, in which the two cytosines at positions -70 and -69 were changed to thymines, did not affect BA-1 binding but reduced MS-1 binding and abolished MS-2R binding (Fig. 7A and B, lanes 4). Mutation M2, carrying a CC-to-TT substitution at positions -73 and -72, abolished binding of MS-2R (Fig. 7A, lane 7) and markedly reduced binding of BA-1 and MS-1 (Fig. 7A and B, lanes 7). Instead, binding complexes with mobilities different from those of BA-1, MS-1, and MS-2R were detected (Fig. 7A and B, lanes 7). The C $\rightarrow$ T mutation (M3) at position -76 abolished MS-1 and MS-2R binding and reduced BA-1 binding (Fig. 7A and B, lanes 10). The M4 mutation, carrying an A $\rightarrow$ G change at position -77, had no effect on MS-2R binding but markedly reduced binding of BA-1 and MS-1 (Fig. 7A and B, lanes 13). These data (summarized in Fig. 7C) were consistent with other results from independent competition experiments, in which the mutated oligonucleotides M1 to M4 were used as cold competitors for wild-type binding of MS-1, BA-1, and

FIG. 7. Identification of DNA contact sites for BA-1, MS-1, and MS-2R. (A) The radiolabeled probes used in each binding assay and the position of mutated nucleotides are shown at the top. Probes were standardized to an identical specific activity and added to either no nuclear extract (lane 1) or to IM9 nuclear extract (1.6 μ g) in the absence (-; lanes 1, 2, 4, 7, 10, and 13) or presence of a 500 molar excess of box A wild-type (WT) unlabeled competitor DNA (lanes 3, 5, 8, 11, and 14) or the unlabeled mutant oligonucleotide competitor DNA (M1, lane 6; M2, lane 9; M3, lane 12; and M4, lane 15). BA-1 and MS-2R DNA-binding activities are marked with arrows. (B) Results of an experiment similar to the one described for panel A, except for the use of U937 nuclear extract (to demonstrate the MS-1 complex). (C) The DNA sequences of the probes used and a summary of their relative in vitro binding activities (ranging from no binding [-] to strong binding [++++] activity). The nucleotide residues replaced in each mutant oligonucleotide and their positions are indicated below the wild-type (WT) sequence.

FIG. 8. Role of BA-1, MS-1, and MS-2R complexes in gene expression. Luciferase reporter gene activity, corrected for transfection efficiency, is expressed as fold activity above the background activity conferred by the promoterless control plasmid pATLuc in uninduced and PMA-induced U937 cells. Histograms demonstrate, respectively, the activities of wild-type (WT) (–81{A | B}–35) and mutants M1 to M4 (see Fig. 7C). Each histogram represents the mean $\pm$ standard deviation (error bar) of two independent transfection experiments (see the text for details).

MS-2R at molar excesses of 100, 250, and 500 (data not shown). To determine the effects of these mutations on gene expression, the M1 to M4 mutations were individually introduced in the –81{A | B}–35 construct, and wild-type and mutant constructs were each transfected into uninduced and PMA-induced U937 and IM9 cells. Gene expression was then quantified by using the luciferase reporter. As can be seen in Fig. 8, the positive loss of the MS-2R-binding site (M1 mutant) had no effect on gene expression in the CD18-positive cell line U937, as predicted given the lack of expression of MS-2R in U937 cells. Interestingly, however, the M1 mutant did not lead to gene expression in the CD18-negative cell line IM9 (data not shown), suggesting that MS-2R is not likely to function as a simple cell-specific repressor. The marked reduction in binding of MS-1 and/or BA-1 to the M2, M3, and M4 mutants also led to a significant reduction in gene expression, indicating direct roles for BA-1 and MS-1 in transcriptional activation of the CD18 gene.

Previous studies showed that the GAGA element in the CD11a promoter, with the core 9-mer motif 5'-GGAG GAAGT, also interacts with BA-1 and MS-1 as well as with a factor homologous to MS2-R (reference 62 and data not shown). Mutational analysis of the GAGA motif showed that the two guanines in the GGAA core are critical for the binding of MS-1 and BA-1 (reference 62 and data not shown). Alignment of the CD11a GAGA sequence with that of box A shows a perfect conservation of the core 9-mer sequence (GGAG GAAGT), which includes a 7-mer (AGGAAGT) present in box B. On the basis of this analysis, we derived a BA-1 consensus sequence, 5'-G/AG/C/AAGGAAG/AT, that is in

agreement with that for GABP as derived by the binding-site selection technique (8). These data, when added to the immunologic cross-reactivity identified here between BA-1 and GABP (Fig. 6), lead to the conclusion that BA-1 and GABP are highly homologous or identical factors. Although the MS-1 element requires the same GGAA core sequence used by BA-1, it can be distinguished from the BA-1 element by its preference for one or two guanines at the –2 and –3 positions (relative to the core GGAA sequence) and its critical requirement for the guanine at the +1 position (see the M3 mutation-binding profile [Fig. 7A and B, lanes 10]), giving the preliminary MS-1 consensus sequence 5'-G/AG/CAGGA AGT. The MS-2R consensus site conforms to the MS-2 consensus sequence (5'-CXXCCT/ACCC) (62 and unpublished observations), except for an adenine instead of the cytosine at the most 3' position. This difference, as well as the different tissue distribution of the ubiquitous MS-2 (62) and MS-2R (that is lacking in U937, CEM, and Jurkat cells but present in IM9 and HeLa cells), suggests that the two factors, while sharing similar DNA-binding motifs, may represent distinct proteins or tissue-specific posttranslational modifications of the same protein.

DISCUSSION

In this study, we have defined the minimal CD18 promoter required for myeloid and lymphoid cell-specific gene expression. This region also confers phorbol ester-induced transcriptional upregulation and encompasses two closely spaced short inverted *cis*-elements, box A and box B, which exhibit cell-specific DNA-protein interactions that correlate with the expression pattern of the gene. Boxes A and B contain the consensus core sequence GGAA/T, the binding site for members of the *ets* family of transcription factors (38). The Ets factors are a diverse group of proteins having in common an 85-amino-acid Ets domain that interacts with a purine-rich core sequence, GGAA/T (38). Known members of this family include Ets-1 (13), Ets-2 (7), Erg (49, 51), Elk-1 and Elk-2 (48), PU.1/Spi-1 (19, 26), Fli-1 (5), the α subunit of GABP (29), SAP-1 (16), Elf-1 (63), PEA-3 (71), *Drosophila melanogaster* E74 (10) and Elg (46), Spi-B (50), and ER71 and ER81 (8). Many members of this family (e.g., Spi-1, Spi-B, Ets-1, Ets-2, Fli-1, Erg, and GABP) are expressed in hematopoietic cells; and some, such as Spi-1, Ets-1 and Fli-1, are involved in the induction of erythroleukemia, suggesting a role for some members of the Ets family in hematopoietic cell growth and differentiation (reviewed in reference 34). Several Ets proteins, including Ets-1, Ets-2, SAP-1, Fli-1, Spi-1, PEA-3, and NRF-2 and E4TF1 (GABP-like factors), have been shown to function as transcriptional activators (25, 34, 67, 70), further supporting a role for Ets proteins in lineage commitment and stage progression during hematopoiesis.

The present data show that at least two members of the Ets family are involved in the tissue-specific and phorbol ester-inducible expression of the CD18 gene in hematopoietic cells. One factor, BA-1, is immunologically related to GABP, a heterodimer that was initially isolated on the basis of its ability to bind the ICP4 promoter in herpes simplex virus type 1 required for immediate early gene activation (29). A similar element has also been found necessary for transcription of the adenovirus E4 gene (70) and of the cytochrome *c* oxidase IV and Vb subunit genes (67). GABP consists of the Ets member GABP α , which can associate with one of two isoforms (β 1 and β 2) of a notch-related β subunit (29, 64). Binding of GABP α to β 1 and β 2 is stabilized by a dimerization domain in β 1 (lacking in β 2) that allows formation of a high-affinity tet-

ramer. Additional putative β isoforms, named $\gamma 1$ and $\gamma 2$, have been recently isolated from HeLa cells (67). Mutational analysis of the BA-1-binding site confirmed the Ets nature of this factor and defined a consensus sequence for its interaction with box A that is essentially identical to that previously derived for GABP by the binding site selection technique (8). Supershift studies suggest that BA-1 is a heteromeric complex consisting of α and β subunits (Fig. 6). Our findings also suggest that, as in the case for GABP, binding of the putative α subunit (BA-1 α) is weak and may be significantly enhanced by the presence of a GABP β -like subunit. The simultaneous binding of BA-1 to the repeated Ets binding motifs present in box A and box B appears to be required for transcriptional activation, as point mutations that interfere with the binding of BA-1 to box A alone markedly reduced gene expression. The two GGAA cores contained within the inverted perfect repeat are 28 bp apart, or approximately 2.8 turns of the DNA helix, suggesting that they may lie on the same face of the helix, permitting formation of a stable heteromeric BA-1 complex. The distance between the BA-1-binding sites is longer than that originally found in the herpes simplex virus ICP4 promoter, in which the GABP-binding tandem repeat is only 2 bp apart (64). Recent data however suggest that significantly longer distances between GABP-binding sites are well tolerated. For example, Virbasius et al. found that the two tandem binding sites of NRF-2, a GABP homolog, can be 20 or 30 bp apart without impairing the ability of NRF-2 to bind to these sites simultaneously (67). No previous examples in which the GABP recognition site is an inverted rather than a tandem repeat are known. Whether the GABP β subunits still allow enough flexibility for the α subunits to bind cooperatively under these conditions remains to be determined.

A second complex, MS-1, detected only in myeloid cells, also forms when nuclear extracts are probed with labeled box A or B oligonucleotides. The Ets nature of MS-1 was confirmed by mutagenesis of the essential biguanidine residues in the GGA core motif as well as the selective competition by the PU.1 consensus sequence, suggesting that MS-1 may be related to PU.1/Spi-1 or Spi-B factors. However, a polyclonal antibody directed against PU.1 did not produce a supershift of the MS-1 complex in PMA-induced U937 cells (Fig. 6), suggesting that MS-1 may be distinct from PU.1. It remains possible that MS-1 is a proteolytic fragment of PU.1, since the anti-PU.1 antibody used in the present study is directed against an N-terminal peptide of PU.1 and the Ets domain in PU.1 is C terminal. It is also possible that MS-1 is related to other Ets factors expressed in hematopoietic cells, such as Spi-B, Fli-1, and Erg, or, given its restricted expression to myeloid cells, that it represents a novel member of the Ets family. Further studies will be required to define the nature of MS-1 and its relationship to other known Ets factors.

In addition to the major complexes BA-1 and MS-1, three other complexes, MS-2R, MS-3, and MS-4, were detected in mobility shift assays using box A and/or B oligonucleotides. MS-2R was detected primarily in the CD18-negative cell lines HeLa and IM9. This complex has a consensus-binding sequence that is similar but not identical to that of MS-2, a yet-to-be-characterized ubiquitous factor that binds to the CD11a, CD11b, CD11c, and CD43 promoters (62, and data not shown). The lack of expression of the wild-type and M1 constructs in the MS-2R-positive but CD18-negative cell line IM9 suggests that this complex plays a minimal role, if any, in regulating tissue-specific CD18 expression. The minor complex MS-3 may be related to MS-1 on the basis of the fact that it has the same cell distribution and oligonucleotide competition profiles. The nature or function of the other minor complex,

MS-4, is unclear at present. Although it has mobility similar to that of MS-3, it does not bind to box A and is found only in lymphoid and HeLa cell lines, suggesting that it is distinct from MS-3 or that it requires tissue-specific posttranslational modifications for binding. The lack of correlation between MS-4 binding and CD18 gene expression suggests that MS-4 may play a minimal role in gene expression.

The mechanism by which interaction of these putative factors with boxes A and B leads to tissue-specific expression and contributes to phorbol ester-inducible expression of the CD18 gene remains speculative at present. Expression of CD18 mirrors the DNase I protection profiles observed *in vitro*, suggesting that the factors that form over the protected elements mediate the tissue-specific expression of the CD18 gene. In T cells, only one major complex, BA-1, forms over the box A and box B elements and is apparently responsible for the DNase I protection seen. In the CD18-negative cell line HeLa, the same complex also forms (confirmed by supershift analysis with anti-GABP α and GABP β antibodies [data not shown]), yet no DNase I protection is seen. Given this scenario, expression of the CD18 gene in T cells but not in HeLa cells may be explained by differences in the composition of the β subunits that associate with BA-1 α , leading in one case to formation of stable complexes and in the others to weak complexes that remain susceptible to DNase I. Similar findings have been recently shown in the case of NRF-2, a GABP homolog. The NRF-2 α subunit did not protect the binding site from DNase I cleavage even when complexed with the $\gamma 1$ or $\gamma 2$ subunits (67). Protection was seen only when the $\alpha\beta 1$ or $\alpha\beta 2$ heterodimers were used. Although HeLa cells contain both the β and γ subunits, quantitative differences or posttranslational modifications in these subunits may preferentially generate weak or unstable DNA-binding complexes, preventing expression of the CD18 gene in this cell line. Alternatively, other members of the β -subunit gene family may be expressed in hematopoietic cell lines and be responsible for the strong binding of BA-1 to box A and box B elements, leading to transcriptional activation. The somewhat higher level of CD18 gene expression in U937 cells than in Jurkat cells may reflect the additional presence of the myeloid-specific factor, MS-1. Transcriptional activation in U937 cells may be enhanced by a simultaneous binding of BA-1 and MS-1 to box A and box B and/or through inter-Ets protein-protein interactions. Although examples of the latter have not yet been described, a recent study has shown that the simultaneous binding of Ets-1 and PU.1/Spi-1, respectively, to the μA and μB elements in the heavy-chain gene enhancer is required to elicit enhancer activity in a tissue-specific manner (36). Analysis of the mechanisms by which interaction of MS-1 and BA-1 may drive myeloid-specific expression will require a detailed characterization of these factors and an assessment of their individual function in a reconstituted system.

The $-81\{A | B\}-35$ construct containing box A and box B elements not only mediated baseline expression of the reporter gene but also conferred phorbol ester inducibility, particularly in Jurkat cells. Interestingly, the PMA-induced activation of other promoters/enhancers has been recently shown to require Ets-1 binding sites. Activation of the stromelysin promoter by PMA requires the integrity of two Ets binding sites within it but is independent of an adjacent AP-1-binding site (69). The phorbol ester induction of the T-cell receptor β -chain gene enhancer is also mediated by two elements which contain binding sites for Ets factors (47). Thus, the Ets family may play a general role in the regulated expression of many genes in response to activation or differentiation signals. PMA treatment of Jurkat and U937 cells led, respectively, to subtle or

significant modifications in the intensity of the BA-1 and MS-1 complexes, reflecting perhaps transcriptional and/or posttranslational modifications that may contribute to the phorbol ester response as has been shown for other Ets factors (18, 31, 43).

As in the case of the CD18 subunit gene, the genes encoding the associated CD11 (α) subunits respond similarly to the same extracellular signals during myeloid and lymphoid differentiation or immune activation, suggesting that similar transcription factors may be involved in the coordinate expression of these genes. Such a coordination is essential for the expression of the CD18 and CD11 gene products as heterodimeric components of a single cell surface membrane receptor. The data presented here and in a previous report (62) reveal some emerging similarities in the organization of the CD11a and CD18 promoters and in the nature of the *trans*-acting factors that drive their concerted expression. The CD11a promoter contains two inverted Ets elements, named the LEU box and the GAGA box, which are 25 bp apart (the distance between the two GGAA cores) and are protected from DNase I cleavage (data not shown). The GAGA box binds MS-1 and MS-2 (62) and BA-1 (data not shown) and contributes largely to the myeloid expression of the CD11a gene. Preliminary data show that the LEU box binds weakly to BA-1. The similarities in the organization of the CD11a and CD18 promoters and in the nature of their *trans*-acting factors may account for the coordinate expression of these distinct genes in hematopoietic cells. The CD18 promoter also shares with the promoters of CD11a, CD11b, CD11c, and other integrins, such as CD41 (α IIb) (31, 45), α 5 (6), and β 1 (11), as well as nonintegrins such as *mb-1* (65), the *src*-related oncogene *p56^{lck}* (32), stromelysin (69), and the pre-B cell λ 5 gene (27), the lack of TATA, CAAT, and initiator elements and initiates transcription at multiple start sites. Sequence analysis of these promoters that direct tissue- or stage-specific gene expression reveals the presence of multiple putative or proven Ets binding sequences. The ability of Ets binding repeats to impart tissue-specific and regulated gene expression, as seen in the CD18 promoter, may reflect the general structure for a new class of promoters that are TATA-less and initiatorless but contain closely spaced Ets repeats, often in the absence of sites for other transcription factors. Recruitment of the general transcription machinery in this case (21) may take place by providing a joint activation domain formed by the concerted binding of the Ets factors or by their recruitment of another activator protein(s).

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REFERENCES

- Agura, E. D., M. Howard, and S. J. Collins. 1992. Identification and sequence analysis of the promoter for the leukocyte integrin β -subunit (CD18): a retinoic acid-inducible gene. *Blood* 79:602-609.
- Arnaout, M. A. 1990. Leukocyte adhesion molecule deficiency: its structural basis, pathophysiology and implications for modulating the inflammatory response. *Immunol. Rev.* 114:145-180.
- Arnaout, M. A. 1990. Structure and function of the leukocyte adhesion molecules CD11/CD18. *Blood* 75:1037-1050.
- Arnaout, M. A., E. Remold-O'Donnell, M. W. Pierce, P. Harris, and D. G. Tenen. 1988. Molecular cloning of the human and guinea pig α subunit of leukocyte adhesion glycoprotein, Mo1. Chromosomal localization and homology to the α subunits of integrins. *Proc. Natl. Acad. Sci. USA* 85:2776-2780.
- Ben-David, Y., E. B. Giddens, K. Letwin, and A. Bernstein. 1991. Erythroleukemia induction by Friend murine leukemia virus: insertional activation of a new member of the *ets* gene family, Fli-1, closely linked to *c-Ets-1*. *Genes Dev.* 5:908-918.
- Birkenmeier, T. M., J. J. McQuillan, E. D. Boedeker, W. S. Argraves, E. Ruoslahti, and D. C. Dean. 1991. The α 5 β 1 fibronectin receptor: characterization of the α 5 gene promoter. *J. Biol. Chem.* 266:20544-20549.
- Boulukos, K. E., P. Pognonec, A. Begue, F. Galibert, J. C. Gesquiere, D. Stehelin, and J. Ghysdael. 1988. Identification in chickens of an evolutionarily conserved cellular *ets-2* gene (*c-ets-2*) encoding nuclear proteins related to the products of the *c-ets* proto-oncogene. *EMBO J.* 7:697-705.
- Brown, T. A., and S. L. McKnight. 1992. Specificities of protein-protein and protein-DNA interaction of GABP α and two newly identified Ets-related proteins. *Genes Dev.* 6:2502-2512.
- Burmeister, M., E. R. Price, T. de Lange, U. Tantravahi, R. M. Myers, and D. R. Cox. 1991. A map of the distal region of the long arm of human chromosome 21 constructed by radiation hybrid mapping and pulsed-field gel electrophoresis. *Genomics* 9:19-30.
- Burtis, K. C., C. S. Thummel, C. W. Jones, F. D. Karim, and D. S. Hogness. 1990. The *Drosophila* 74EF early puff contains E74, a complex ecdysone-inducible gene that encodes two *ets*-related proteins. *Cell* 61:85-99.
- Cervella, P., L. Silengo, C. Pastore, and F. Altruda. 1993. Human β 1-integrin gene expression is regulated by two promoter regions. *J. Biol. Chem.* 268:5148-5155.
- Chen, H.-M., H. L. Pahl, R. J. Scheibe, D.-E. Zhang, and D. G. Tenen. 1993. The Sp1 transcription factor binds the CD11b promoter specifically in myeloid cells *in vivo* and is essential for myeloid-specific promoter activity. *J. Biol. Chem.* 268:8230-8239.
- Chen, J. H. 1985. The protooncogene *c-ets* is preferentially expressed in lymphoid cells. *Mol. Cell Biol.* 5:2993-3000.
- Corbi, A. L., R. S. Larson, T. K. Kishimoto, T. A. Springer, and C. C. Morton. 1988. Chromosomal location of the genes encoding the leukocyte adhesion receptors LFA-1, Mac-1 and p150,95. Identification of a gene cluster involved in cell adhesion. *J. Exp. Med.* 167:1597-1607.
- Cornwell, R. D., K. A. Gollahon, and D. D. Hickstein. 1993. Description of the leukocyte function-associated-antigen-1 (LFA1 or CD11a) promoter. *Proc. Natl. Acad. Sci. USA* 90:4221-4225.
- Dalton, S., and R. Treisman. 1992. Characterization of SAP-1, a protein recruited by serum response factor to the *c-fos* serum response element. *Cell* 68:597-612.
- Dignam, J. D., R. M. Lebovitz, and R. G. Roeder. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* 11:1475-1489.
- Gille, H., A. D. Sharrocks, and P. E. Shaw. 1992. Phosphorylation of transcription factor p62 by MAP kinase stimulates ternary complex formation at *c-fos* promoter. *Nature (London)* 358:414-417.
- Goebel, M. G., F. Moreau-Gachelin, D. Ray, P. Tombourin, A. Tavittian, M. J. Klemsz, S. R. McKercher, A. Celada, C. Van Beveren, and R. A. Maki. 1990. The PU.1 transcription factor is the product of the putative oncogene Spi.1. *Cell* 61:1165-1166.
- Gorman, C. M., L. F. Moffat, and B. H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyl transferase in mammalian cells. *Nucleic Acids Res.* 2:1044-1051.
- Hagemeier, C., A. J. Bannister, A. Cook, and T. Kouzarides. 1993. The activation domain of transcription factor PU.1 binds the retinoblastoma (RB) protein and the transcription factor TFIID *in vitro*: RB shows sequence similarity to TFIID and TFIIB. *Proc. Natl. Acad. Sci. USA* 90:1580-1584.
- Havney, J. C., N. J. Peffer, J. E. Bisi, S. A. Thomson, R. Cadilla, J. A. Josey, D. J. Ricca, C. F. Hassman, M. A. Bonham, K. G. Au, S. G. Carter, D. A. Bruckenstein, A. L. Boyd, S. A. Noble, and L. E. Babiss. 1992. Antisense and antigene properties of peptide nucleic acids. *Science* 258:1481-1485.
- Hickstein, D. D., A. L. Back, and S. J. Collins. 1988. Regulation of expression of the CD11b and CD18 subunits of the neutrophil adherence receptor during human myeloid differentiation. *J. Biol.*

- Chem. **264**:21812-17.
24. Hogge, D. E., S. Misawa, N. Z. Parsa, A. Pollak, and J. R. Testa. 1984. Abnormalities of chromosome 16 in association with acute myelomonocytic leukemia and dysplastic bone marrow eosinophils. *J. Clin. Oncol.* **2**:550-557.
 25. Klemsz, M. J., R. A. Maki, T. Papayannopoulou, J. Moore, and R. Hromas. 1993. Characterization of the *ets* oncogene family member, *fli-1*. *J. Biol. Chem.* **268**:5769-5773.
 26. Klemsz, M. J., S. R. McKercher, A. Celada, C. Van Beveren, and R. A. Maki. 1990. The macrophage and B cell-specific transcription factor PU.1 is related to the *ets* oncogene. *Cell* **61**:113-24.
 27. Kudo, A., N. Sakaguchi, and F. Melchers. 1987. Organization of the murine Ig-related 15 locus of the mouse, which appears transcribed selectively in pre-B lymphocytes. *EMBO J.* **6**:103-107.
 28. Kumar, V., and P. Chambon. 1988. The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer. *Cell* **55**:145-156.
 29. LaMarco, K., C. C. Thompson, B. P. Byers, E. M. Walton, and S. L. McKnight. 1991. Identification of Ets- and notch-related subunits in GA binding protein. *Science* **253**:789-92.
 30. LeBeau, M. M., L. A. Larson, M. A. Bitter, J. W. Vardiman, H. M. Golomb, and J. D. Rowley. 1984. Association of an inversion of chromosome 16 with abnormal marrow eosinophils in acute myelomonocytic leukemia: a unique cytogenetic-clinicopathologic association. *N. Engl. J. Med.* **309**:630-636.
 31. Lemarchandel, V., J. Ghysdael, V. Mignotte, C. Rahuel, and P.-H. Romeo. 1993. GATA and Ets *cis*-acting sequences mediate megakaryocyte-specific expression. *Mol. Cell. Biol.* **13**:668-676.
 32. Leung, S., S. McCracken, J. Ghysdael, and N. G. Miyamoto. 1993. Requirement of an ETS-binding element for transcription of the human *lck* type I promoter. *Oncogene* **8**:989-997.
 33. Lopez-Cabrera, M., A. Nueda, A. Vara, J. Garcia-Aguilar, A. Tugores, and A. L. Corbi. 1993. Characterization of the p150,95 leukocyte integrin α subunit (CD11c) gene promoter: identification of *cis*-acting elements. *J. Biol. Chem.* **268**:1187-1193.
 34. Macleod, K., D. LePrince, and D. Stehelin. 1992. The *ets* gene family. *Trends Biochem. Sci.* **17**:251-256.
 35. Miller, J. M., R. Schwarting, and T. A. Springer. 1986. Regulated expression of the Mac-1, LFA-1, p150,95 glycoprotein family during leukocyte differentiation. *J. Immunol.* **137**:2891-2900.
 36. Nelsen, B., G. Tian, B. Erman, J. Gregoire, R. Maki, B. Graves, and R. Sen. 1993. Regulation of lymphoid-specific immunoglobulin μ heavy chain gene enhancer by ETS-domain proteins. *Science* **261**:82-86.
 37. Nelson, C., H. Rabb, and M. A. Arnaout. 1992. Genetic cause of leukocyte adhesion molecule deficiency: abnormal splicing and a missense mutation in a conserved region of CD18 impair cell surface expression of $\beta 2$ integrins. *J. Biol. Chem.* **267**:3351-3357.
 38. Nye, J. A., J. M. Petersen, C. V. Gunther, M. D. Jonsen, and B. J. Graves. 1992. Interaction of murine Ets-1 with GGA-binding sites establishes the ETS domain as a new DNA-binding motif. *Genes Dev.* **6**:975-90.
 39. Pahl, H. L., A. G. Rosmarin, and D. G. Tenen. 1992. Characterization of the myeloid-specific CD11b promoter. *Blood* **79**:865-70.
 40. Pahl, H. L., R. J. Scheibe, D.-E. Zhang, H.-M. Chen, D. L. Galson, R. A. Maki, and D. G. Tenen. 1993. The proto-oncogene PU.1 regulates expression of the myeloid-specific CD11b promoter. *J. Biol. Chem.* **268**:5014-5020.
 41. Patarroyo, M. 1992. CD11/CD18 (Leu-CAMs, $\beta 2$ integrins) and CD54 (ICAM-1): identification as adhesion molecules and relevance to leukocyte physiology, p. 165-182. *In* C. G. Gahmberg, T. Mandrup-Poulsen, L. Wogensen Bach, and B. Hokfelt (ed.), *Leukocyte adhesion: basic and clinical aspects*. Novo Nordisk Foundation Symposium, vol. 6.
 42. 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 κ 3' enhancer activity. *Mol. Cell. Biol.* **12**:368-378.
 43. 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.
 44. Potter, H., L. Weir, and P. Leder. 1984. Enhancer-dependent expression of human kappa immunoglobulin genes introduced into mouse pre-B lymphocytes by electroporation. *Proc. Natl. Acad. Sci. USA* **81**:7161-7165.
 45. Prandini, M. H., E. Denarier, P. Frachet, G. Uzan, and G. Marguerie. 1988. Isolation of the human glycoprotein IIb gene and characterization of the 5' flanking region. *Biochem. Biophys. Res. Commun.* **156**:595-601.
 46. Pribyl, L. J., D. K. Wtason, R. A. Schulz, and T. S. Papas. 1991. D-*elg*, a member of the Drosophila *ets* gene family: a sequence, expression and evolutionary comparison. *Oncogene* **6**:1175-1183.
 47. Prosser, H. M., D. Wotton, A. Gegonne, J. Ghysdael, S. Wang, N. A. Speck, and J. J. Owen. 1992. A phorbol ester response element within the human T-cell receptor β -chain enhancer. *Proc. Natl. Acad. Sci. USA* **89**:9934-9938.
 48. Rao, V. N., K. Huebner, M. Isobe, A. Ar-Rushdi, C. M. Croce, and E. S. P. Reddy. 1989. ELk, tissue-specific *ets*-related genes on chromosomes X and 14 near translocation breakpoints. *Science* **244**:66-70.
 49. Rao, V. N., T. S. Papas, and E. S. P. Reddy. 1987. *erg*, a human *ets*-related gene on chromosome 21: alternative splicing, polyadenylation, and translation. *Science* **237**:635-639.
 50. Ray, D., R. Bosselut, J. Ghysdael, M.-G. Mattei, A. Tavittian, and F. Moreau-Gachelin. 1992. Characterization of Spi-B, a transcription factor related to the putative oncoprotein Spi-1/PU.1. *Mol. Cell. Biol.* **12**:4297-4304.
 51. Reddy, E. S., and V. N. Rao. 1991. *erg*, an *ets*-related gene, codes for sequence-specific transcriptional activators. *Oncogene* **6**:2285-9.
 52. Roberts, R., J. Gallagher, E. Spooner, T. D. Allen, F. Bloomfield, and T. M. Dexter. 1988. Heparan sulphate-bound growth factors; a mechanism for stromal cell mediated haemopoiesis. *Nature (London)* **332**:376-378.
 53. Roesler, W., G. Vandenbark, and R. Hanson. 1988. Cyclic AMP and the induction of eukaryotic gene transcription. *J. Biol. Chem.* **263**:9063-9066.
 54. Rosenthal, P., I. J. Rimm, T. Umiel, J. D. Griffin, R. Osathanondh, S. F. Schlossman, and L. M. Nadler. 1983. Ontogeny of human hematopoietic cells: analysis utilizing monoclonal antibodies. *J. Immunol.* **31**:232-237.
 55. Rosmarin, A. G., R. Levy, and D. G. Tenen. 1992. Cloning and analysis of the CD18 promoter. *Blood* **79**:2598-2604.
 56. Rosmarin, A. G., S. Weil, S. K. Law, J. D. Griffin, M. A. Arnaout, and D. G. Tenen. 1988. Differential expression of Mol and myeloperoxidase genes during myeloid differentiation. *Blood* **73**:131-136.
 57. Rubin, C. M., R. A. Larson, M. A. Bitter, J. J. Carrino, M. M. Le Beau, M. O. Diaz, and J. D. Rowley. 1987. Association of a chromosomal 3;21 translocation with the blast phase of chronic myelogenous leukemia. *Blood* **70**:1338-1342.
 58. Ryseck, R. P., and R. Bravo. 1991. c-JUN, JUN B and JUN D differ in their binding affinities to AP-1 and CRE consensus sequence: effect of FOS proteins. *Oncogene* **6**:533-542.
 59. Sacchi, N., J. Nalbantoglu, F. R. Sergovich, and T. S. Papas. 1988. Human ETS2 gene on chromosome 21 is not rearranged in Alzheimer disease. *Proc. Natl. Acad. Sci. USA* **85**:7675-7679.
 60. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463-5467.
 61. Shelley, C. S., and M. A. Arnaout. 1991. The promoter of the CD11b gene directs myeloid-specific and developmentally regulated expression. *Proc. Natl. Acad. Sci. USA* **88**:10525-10529.
 62. Shelley, C. S., O. C. Farokhzad, and M. A. Arnaout. 1993. Identification of cell-specific and developmentally regulated nuclear factors which direct myeloid and lymphoid expression of the CD11a gene. *Proc. Natl. Acad. Sci. USA* **90**:5364-5368.
 63. Thompson, C. B., C.-Y. Wang, I.-C. Ho, P. R. Bohjanen, B. Petryniak, C. H. June, S. Miesfeldt, L. Zhang, G. J. Nabel, B. Karpinski, and J. M. Leiden. 1992. *cis*-acting sequences required for inducible interleukin-2 enhancer function bind a novel Ets-related protein, Elf-1. *Mol. Cell. Biol.* **12**:1043-1053.
 64. Thompson, C. C., T. A. Brown, and S. L. McKnight. 1991. Convergence of Ets- and notch-related structural motifs in a heteromeric DNA binding complex. *Science* **253**:762-768.

65. **Travis, A., J. Hagman, and R. Grosschedl.** 1991. Heterogeneously initiated transcription from the pre-B- and B-cell-specific mb-1 promoter: Analysis of the requirement for upstream factor-binding sites and initiation site sequences. *Mol. Cell. Biol.* **11**:5756–5766.
66. **Urness, L. D., and C. S. Thummel.** 1990. Molecular interactions within the ecdysone regulatory hierarchy: DNA binding properties of the *Drosophila* ecdysone-inducible E74A protein. *Cell* **63**:47–61.
67. **Virbasius, J. V., C.-M. A. Virbasius, and R. C. Scarpulla.** 1993. Identity of GABP with NRF-2, a multisubunit activator of cytochrome oxidase expression, reveals a cellular role of an ETS domain activator of viral promoters. *Genes Dev.* **7**:380–392.
68. **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**:191–3.
69. **Wasylyk, C., A. Gutman, R. Nicholson, and B. Wasylyk.** 1991. The c-Ets oncoprotein activates the stromelysin promoter through the same elements as several nonnuclear oncoproteins. *EMBO J.* **10**:1127–1134.
70. **Watanabe, H., J.-I. Sawada, K.-I. Yano, K. Yamaguchi, M. Goto, and H. Handa.** 1993. cDNA cloning of transcription factor E4TF1 subunits with Ets and notch motifs. *Mol. Cell. Biol.* **13**:1385–1391.
71. **Xin, J. H., A. Cowie, P. Lachance, and J. A. Hassell.** 1992. Molecular cloning and characterization of PEA3, a new member of the Ets oncogene family that is differentially expressed in mouse embryonic cells. *Genes Dev.* **6**:481–96.
72. **Zhang, Y., and W. N. Rom.** 1993. Regulation of the interleukin-1 β (IL-1 β) gene by mycobacterial components and lipopolysaccharide is mediated by two nuclear factor-IL6 motifs. *Mol. Cell. Biol.* **13**:3831–3837.