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An AML-1 consensus sequence binds an osteoblast-specific complex and transcriptionally activates the osteocalcin gene

(core-binding factor/polyomavirus enhancer binding protein 2/runt domain/bone specific/osteocalcin box II)

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ABSTRACT **Tissue and cell-type specific expression of the rat osteocalcin (rOC) gene involves the interplay of multiple transcriptional regulatory factors. In this report we demonstrate that AML-1 (acute myeloid leukemia-1), a DNA-binding protein whose genes are disrupted by chromosomal translocations in several human leukemias, interacts with a sequence essential for enhancing tissue-restricted expression of the rOC gene. Deletion analysis of rOC promoter-chloramphenicol acetyltransferase constructs demonstrates that an AML-1-binding sequence within the proximal promoter (−138 to −130 nt) contributes to 75% of the level of osteocalcin gene expression. The activation potential of the AML-1-binding sequence has been established by overexpressing AML-1 in osteoblastic as well as in nonosseous cell lines. Overexpression not only enhances rOC promoter activity in osteoblasts but also mediates OC promoter activity in a nonosseous human fibroblastic cell line. A probe containing this site forms a sequence specific protein–DNA complex with nuclear extracts from osteoblastic cells but not from nonosseous cells. Antisera supershift experiments indicate the presence of AML-1 and its partner protein core-binding factor β in this osteoblast-restricted complex. Mutations of the critical AML-1-binding nucleotides abrogate formation of the complex and strongly diminish promoter activity. These results indicate that an AML-1 related protein is functional in cells of the osteoblastic lineage and that the AML-1-binding site is a regulatory element important for osteoblast-specific transcriptional activation of the rOC gene.**

Osteocalcin (OC), a developmentally regulated bone specific marker of osteoblast differentiation, is an established model for examining molecular mechanisms that control and maintain the osteoblastic phenotype. Several cis-acting elements as well as components of nuclear architecture (chromatin organization and nuclear matrix) regulate transcription of this gene (1). Bidwell *et al.* (2) identified sites in the rat OC (rOC) promoter that interact with an osteoblast-restricted and nuclear-matrix associated DNA-binding complex (nuclear matrix protein-2). This protein–DNA complex interacts with sequence specificity with three sites in the rOC promoter: sites A and B (nt −604 to nt −599 and nt −440 to nt −435) flanking the vitamin D receptor-binding element (2) and site C in the proximal promoter (nt −136 to nt −130) (3). The consensus DNA-binding sequence for nuclear matrix protein-2 is PuACCPuCT which is similar to the consensus binding motif Pu/TACCPu/TCA of the recently described transcription factor acute myeloid leukemia-1 (AML-1) (4). Recent studies indicate that the proximal promoter of the mouse OC gene (which bears considerable homology to the rOC gene) contains an osteoblast-specific element designated OSE2 (5) which is homologous to site C. The purpose of this study was to investigate the functional role of the site C nuclear matrix protein-2-binding site in the regulation of rOC gene expression.

AML-1, a transcription factor studied for its involvement in leukemogenesis, is related to the murine enhancer core-binding factor (CBF) (6, 7) and polyomavirus enhancer core-binding protein (PEBP2) (8, 9). Transcriptionally active forms of these factors bind to several viral enhancers (8, 10–14) and promoters (15, 16) at the sequence 5′-TGT/cGGT-3′ or 5′-Pu/TACCPuCA-3′ (4, 6, 8, 17) and are involved in activation of various cell-type restricted genes. These factors consist of two unrelated subunits: α (AML-1/CBFα) containing an evolutionarily conserved runt homology domain which encompasses the DNA-binding and heterodimerization domains of these factors (4, 9, 18) and β (CBFβ), a nonDNA-binding auxiliary factor that heterodimerizes with the α subunits and augments DNA binding (7, 19, 20). All AML-1 family members produce multiple alternatively spliced transcripts that code for multiple isoforms in a cell-type dependent manner (7, 20, 21, 22). These isoforms possess differential transactivation potentials (20, 22) and transcriptionally regulate cell-type restricted or tissue-specific expression of T-cell, B-cell, and myeloid-specific genes (9, 23–26). CBFβ also produces alternatively spliced transcripts but has a ubiquitous distribution (7, 18, 19). The role of AML-1 as a leukemogenic factor and its association with hematopoiesis makes it an excellent candidate for a role in the transcription of the bone specific OC gene.

In this report we show that an AML-1-binding sequence in the proximal rOC promoter functions as an enhancer element and contributes to 75% of the transcriptional activity of the gene. We further show that AML-1 and CBFβ are components of an osteoblast-specific complex that binds to this element. Overexpressed AML-1 transactivates OC gene expression through this element in osteoblastic as well as nonosseous cells. Thus, our investigations demonstrate that an AML-1 related protein plays a key regulatory role in the control of osteoblast-restricted gene expression.

MATERIALS AND METHODS

Cells and Cell Culture. Normal diploid osteoblasts obtained from fetal rat calvariae were isolated as described (27). Rat osteosarcoma cell lines ROS 17/2.8 (27), ROS 25/1 (28), rat osteogenic sarcoma line UMR-106 (29), and human osteosarcoma cell line Saos-2 (American Type Culture Collection) were maintained as described. Nonosseous cell lines include rat embryo fibroblastic line R2 (30) and human fibroblastic

Abbreviations: rOC, rat osteocalcin; AML-1, acute myeloid leukemia-1; CBF, core-binding factor; PEBP, polyomavirus enhancer binding protein; OBSC, osteoblast specific complex; CAT, chloramphenicol acetyltransferase; TK, thymidine kinase; OC Box II, osteocalcin Box II; WT, wild type.

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Table 1. Oligonucleotides

Name		Sequence (5′ strand)	
WT	−151	GCAGCTGCAGTCACC**AACCACA**GCATCC	−124
C1		GCAGCTGCA*tgtc*CC**AACCACA**GCATCC	
CM1		GCAGCTGCAGTCA***gatg*CCACA**GCATCC	
AML-1 consensus		CGAGTAT**TGTGGT**TAATACG	
C-MET	−162	CCTTCGCCCCGGCAGCTGCAGTCACCAACCACAGC	−128

Wild-type (WT) rOC promoter sequence (nt −151 to −124) containing an AP-1 like TGF-β1 responsive element (32) underlined and AML-1 binding motif in boldface type is shown. Because AP-1 affects rOC gene expression (33), C1 was designed with a mutated AP-1 binding site (italic type). CM1 contains mutated AML-1-binding site (boldface italic type). AML-1-binding site in boldface type is AML-1 consensus (4) oligonucleotide. C-MET, a wild-type rOC promoter sequence containing the AML-1-binding site was used for methylation interference studies.

line IMR-90 (obtained from the National Institute on Aging Cell Repository) and were maintained according to specifications. Proliferating day 3 (d3) and confluent day 10 (d10) cells (mineralized day 16 in the case of primary osteoblasts) were used for preparing nuclear extracts.

Plasmids and Deletion Mutants. 5′ deletion constructs (see Table 2) were produced by progressive shortening of −1097 nt to +23 nt rOC promoter-chloramphenicol acetyltransferase (CAT) construct pOCZCAT (31) from the 5′ end. pGEMCAT is the promoterless control. Double-stranded wild-type (WT), C1, and CM1 oligonucleotides (Table 1) were fused upstream of the promoters of −108CAT construct or herpes simplex thymidine kinase (TK) promoter CAT plasmid pBLCAT2 to produce a series of constructs with one or two copies of the oligonucleotide in either orientation (see Table 3). Expression plasmid containing cDNA of AML-1B, the transactivating form of AML-1 (20), was used for overexpression studies.

***In Vitro* Translation Studies.** Subunits α and β of the murine transcription factor PEBP2 were *in vitro* transcribed and translated from pKSαB1 (26) and pSKHgβ2 (19), respectively, using the TnT T7/T3 Coupled Reticulocyte Lysate System (Promega) according to the manufacturer's specifications.

Analysis of Protein–DNA Interactions. Nuclear extracts were prepared as described (34) using 0.45 M KCl for extraction. Gel-mobility shift (31) and methylation interference (35, 36) assays were performed as described. Protein–DNA complexes from 4 μg of protein, 1 μg of poly(dI-dC)·poly(dI-dC) and 10 fmol of the radiolabeled probe were resolved on 4% nondenaturing gels in Tris/glycine/EDTA buffer at 200 V for 2 h. Antisera supershift experiments contained AML-1 polyclonal antiserum directed to 17 N-terminal amino acids of AML-1 (4), CBFβ (human) antiserum, or preimmune serum (control). For peptide competition, specific antigenic peptide of AML-1 was used (4). Gels were dried and exposed to Amersham Hyperfilm at -70°C for 12 h, except for Fig. 4*A* (36 h).

Transient Transfection Assays and Overexpression Studies. ROS 17/2.8 cells were transiently transfected as described (31) by the DEAE–dextran method (35). For overexpression studies, 7–10 μg of the reporter plasmid was cotransfected with 750 ng-1 μg of the expression plasmid and 500 ng of pRSV-luc (internal control). The amount of total DNA was maintained at 15 μg/100 mm plate with salmon sperm DNA (Sigma, moleculary biology grade). IMR-90 cells were transfected by the $CaCl_2$-HEBES method (35). Cells were harvested from 48 to 60 h posttransfection and CAT activity was determined as described (35). All experiments were done from three to eight times in triplicate with two or more different DNA preparations.

RESULTS

Functional Analysis of the Three AML-1-Binding Sequences in the rOC Promoter. A series of 5′ promoter deletion mutants was used to study the functional contribution of the three AML-1-binding sites to expression of the rOC gene (Table 2). The pOCZCAT construct encompasses all three sites (site A: −604 to −599; site B: −440 to −435; and site C: −136 to −130). As shown in Table 2, elimination of the two upstream sites does not have major functional consequence on the level of gene expression. However, promoter activity is diminished 75% upon elimination of the −165 to −108 fragment containing the site C AML-1-binding sequence. This result led us to focus on the significance of the site C AML-1-binding sequence in the regulation of rOC gene expression.

The Site C AML-1-Binding Sequence Binds a Tissue-Restricted Complex. Gel-mobility shift assays comparing nuclear matrix and nuclear extracts with the site A (2) probe exhibited complexes of higher mobility with the nuclear matrix preparations (3). This difference led us to examine whether the osteoblast-specific property of nuclear matrix complexes (2) was extended to nuclear extracts. Probe C1 encompassing site C (nt −151 to nt −124) formed one major protein–DNA complex (complex A) with osteoblast-derived cells (Fig. 1). Post-proliferative ROS 17/2.8 cells (lane 4), differentiated normal osteoblasts (lane 2), and Saos-2 cells (lanes 5 and 6) also showed another complex of higher mobility and increased intensity (complex B). However, neither complex was detected in gel-binding assays with nuclear extracts from nonosseous cells (lanes 11–14). Since complex A is common to all the osteoblastic cell lines tested, we have designated this nuclear extract-specific complex OBSC (Osteoblast-Specific Complex).

OBSC-Binding Site Resembles the AML-1-Binding Motif and Binds Recombinant AML-1 Protein. Methylation interference analysis of OBSC (Fig. 2 *A*, *B*, and *C*) and complex B (data not shown) identified the protein–DNA binding motifs of the two complexes as 5′-CCAACCAC-3′ and 5′-AACCAC-3′, respectively. These binding sequences closely resemble the DNA-binding motif 5′-Pu/TACCPu/TCA-3′ for the related transcription factors AML-1 (4), CBFα(6), and SEF-1 (14) indicating that the AML-1 related protein(s) constitute the DNA-binding component of the complexes. Consistent with previous reports, complex B may represent a proteolytic cleavage product or a different isoform of an AML-1-like protein (4, 7, 9, 21). Based on its significant contribution to

Table 2. rOC promoter deletion analysis

Deletion construct	Extent of promoter	Promoter activity
pOCZCAT	−1097 – +24	100 ± 4.0
−531CAT	−531 – +24	176 ± 4.5
−348CAT	−348 – +24	159 ± 3.7
−165CAT	−165 – +24	145 ± 2.4
−108CAT	−108 – +24	39 ± 1.9
pGEMCAT	none	2 ± 0.02

Results of transient transfection of ROS 17/2.8 cells with rOC promoter deletion–CAT constructs. CAT activities relative to pOCZCAT are indicated. Data represent mean ± SD of 4–8 experiments.

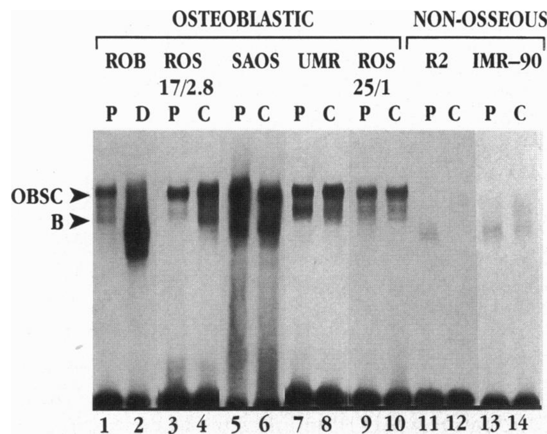

FIG. 1. Gel-mobility shift assays of osteoblastic and nonosseous cell lines. Probe C1 was used in gel- mobility shift assays with proliferating d3 (lanes 3, 5, 7, 9, 11, and 13; indicated by P) and confluent d10 (lanes 4, 6, 8, 10, 12, and 14; indicated by C) nuclear extracts from the cell lines indicated. Nuclear extracts from normal diploid osteoblasts were obtained from proliferating d4 (lane 1) and mineralized d16 (lane 2; indicated by D) cells. Complex A (OBSC) and complex B are indicated.

transcription, conservation of the sequence in other OC promoters (5) and binding to a tissue-specific complex, we designate this OBSC-binding site (nt −138 to nt −130) as the Osteocalcin Box II (OC Box II).

In vitro translated PEBP2α (mouse homologue of AML-1) bound to the WT oligomer which contains OC Box II and formed two distinct complexes (Fig. 2*D*, lane 2; complexes α) in gel-mobility shift assays. When the nonDNA-binding component PEBP2β (mouse homologue of CBFβ) was added to the α subunit in the binding reaction, a complex of slower mobility and higher intensity was formed (lane 3; complex $\alpha+\beta$), indicating that the OC Box II sequence is sufficient for binding AML-1 and its partner protein. No DNA-binding was observed (Fig. 2*E*) with the mutant probe CM1 in which 4 bp CCAA at the 5′ end of the OBSC footprint are substituted (see Table 1).

Characterization of Protein–DNA Interactions Involving OBSC. The binding specificity of OBSC was examined using *in vitro* oligonucleotide competition experiments. Gel-mobility shift analysis with probe C1 (Fig. 3*A*) and AML-1 consensus oligonucleotide (Fig. 3*C*) demonstrated that OBSC and complex B were competed by molar excesses of both the unlabeled C1 oligonucleotide (lanes 3) and the AML-1 consensus sequence (lanes 4), but not by the mutant oligonucleotide CM1 (lanes 2). Protein–DNA interaction was virtually abolished when CM1 (mutant oligomer) was used as the probe (Fig. 3*B*) indicating that the sequence 5′-CCAA-3′ is critical for formation of OBSC. Interestingly, only a 20-fold molar excess of the unlabeled AML-1 consensus oligonucleotide prevented DNA-binding completely (data not shown). Results from these cross-competition experiments indicate that a factor(s) that bind specifically to the AML-1 consensus sequence comprise the DNA-binding component of OBSC.

AML-1 and CBFβ Antisera Partially Supershifts OBSC. To characterize OBSC further, AML-1 (human) antiserum was used in gel- supershift assays with nuclear extracts from an OC-producing rat osteosarcoma cell line ROS 17/2.8 (Fig. 4*A*) and human osteosarcoma cell line Saos-2 (Fig. 4*B*) that does not produce OC. Nuclear extracts from both cell lines show a supershift of OBSC (Fig. 4 *A* and *B*, lanes 4, 5, and 6). The specific antigenic peptide prevents the formation of supershift completely (Fig. 4 *A* and *B*, lanes 7 and 8). CBFβ antiserum

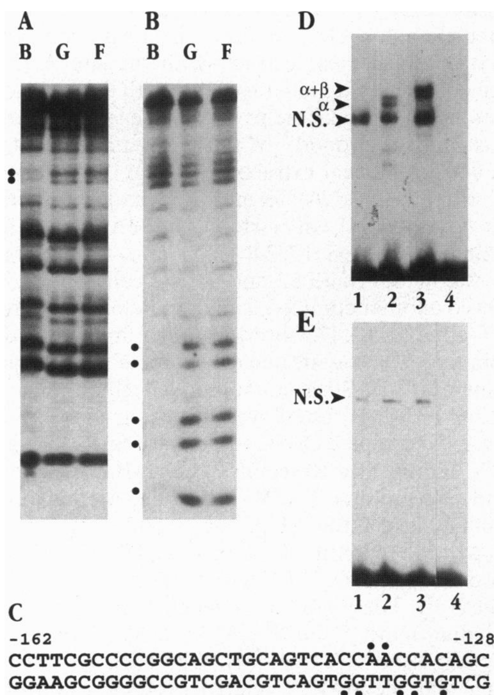

FIG. 2. The OBSC-binding sequence contains an AML-1-binding site. (*A*, *B*, and *C*) Methylation interference analysis. ^{32}P end-labeled 5′ strand (*A*) and 3′ strand (*B*) of the oligonucleotide C-MET (see Table 1) were methylated by dimethylsulfate and used in gel-mobility shift assays with nuclear extracts from d10 ROS 17/2.8 cells. After piperidine cleavage of the probes, equal counts of free probe (lanes F), bound probe (lanes B), and unreacted probe (lanes G) were separated on a 12% sequencing gel. Methylation of G and A residues indicated by filled circles were interfered by OBSC binding. (*C*) Protein–DNA contact points on the double-stranded C-MET oligonucleotide are indicated (filled circles). (*D* and *E*) Binding of *in vitro* translated PEBP2αB1 and PEBP2β proteins to the WT and mutant probes (−151 nt to −124 nt). Proteins were incubated with 10 fmol of radiolabeled probe and separated on a 4% nondenaturing PAGE. αB1 protein (3 μl) (lanes 2) and 3 μl each of αB1 and β proteins (lanes 3) were incubated with a WT probe (*D*) and mutant probe CM1 (*E*). Lanes 1 contain vector (control) and lanes 4 contain the probes alone. Complexes α (lane 2) and $\alpha+\beta$ (lane 3) are indicated in *D*. N.S. indicates a nonspecific band.

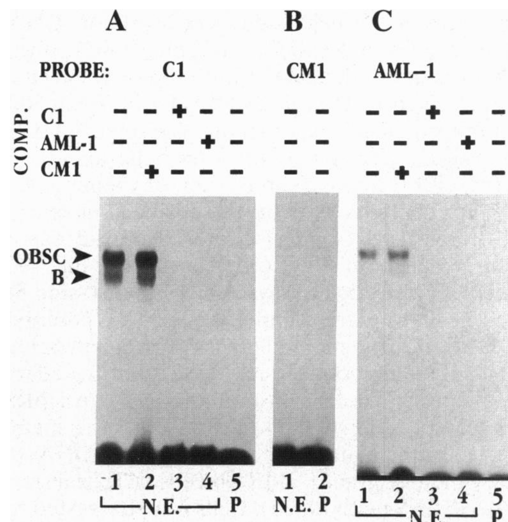

FIG. 3. *In vitro* oligonucleotide competition of OBSC binding. Control oligonucleotide C1 (*A*), mutant oligonucleotide CM1 (*B*), and AML-1 consensus oligonucleotide (*C*) were radiolabeled and used as probes in gel-mobility shift assays with d10 ROS 17/2.8 nuclear extracts (indicated by N.E.). Binding assays were performed as described in the absence or presence of 100-fold molar excesses (1 pmol) of unlabeled double-stranded oligonucleotide as competitors (C1, CM1, or AML-1 consensus; indicated as comp.). P indicates probe alone. Complex A (OBSC) and complex B are indicated.

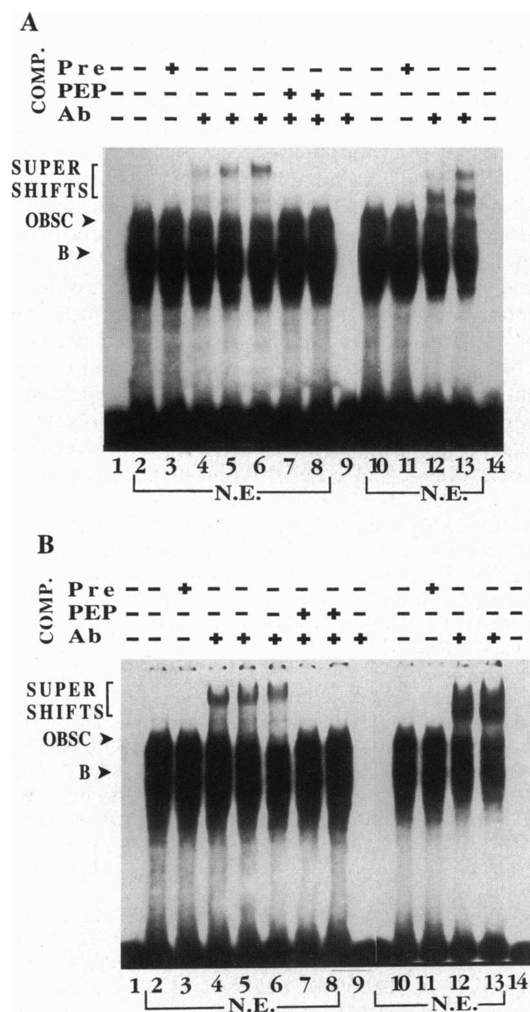

FIG. 4. Supershift-mobility assays of the OBSC complex. ROS 17/2.8 (*A*) and Saos-2 (*B*) nuclear extracts (N.E.) were incubated with increasing amounts of anti-AML-1 (lanes 4, 3 μl; lanes 5, 6 μl; lanes 6, 8 μl) and anti-CBFβ antibodies (lanes 12, 3 μl and lanes 13, 5 μl) in binding reactions with probe C1. Eight micrograms (lanes 7) and 12 μg (lanes 8) of the specific antigenic peptide (indicated as PEP) were added with 3 μl (lanes 7) and 5 μl (lanes 8) of AML-1 antisera, respectively, for peptide competition. Lanes 3 and 13, nuclear extracts incubated with 6 μl of preimmune serum (indicated as Pre). Lanes 9, AML-1 antibody and lanes 14, CBFβ antibody were incubated with probe only. Lanes 1 contain probe alone. Dried gels were exposed for 36 h (*A*) or 12 h (*B*). OBSC, complex B and the supershifted bands are indicated.

was used in similar assays to determine whether OBSC also contain CBFβ, the nonDNA-binding partner protein of AML-1. Both ROS 17/2.8 and Saos-2 nuclear extracts (Fig. 4 *A* and *B*, lanes 12 and 13) show two supershifted bands that probably originate from each of the two complexes, OBSC and complex B. These results indicate that both AML-1 and CBFβ constitute part of the OBSC in osteoblastic cells regardless of OC production. The quantitative difference in the supershift complexes between rat and human osteosarcoma may indicate that the AML-1 (human) antibody possesses limited cross-reactivity to the rat osteosarcoma AML-related protein.

Functional Characterization of OC Box II. Since the AML-1 binding-site is present in several enhancers, we tested whether the functional properties of OC Box II were consistent with the properties of an enhancer element. Table 3 shows that both in homologous and heterologous promoters, presence of the WT oligomer (either orientation) or the C1 oligomer stimulated CAT gene expression by 3–4 fold (compared to −108CAT and pBLCAT2 controls) and the effect was additive upon inclusion of additional copies of the element in either orientation. The mutant constructs CM1-CAT and CM1-tkCAT, however, did not support an increase in the level of rOC gene expression above the controls. These data indicate that OC Box II behaves as an orientation-independent enhancer-like element.

Table 3. Effect of wild-type and mutated OC Box II on homologous and heterologous promoters

Construct	Promoter	Oligonucleotide (no. of copies) and orientation	Relative CAT activity
−108CAT	−108 rOC	none	100 ± 0.5
WT–CAT	−108 rOC	WT (1) correct	408 ± 23
WT(R)–CAT	−108 rOC	WT (1) reverse	416 ± 39
WT(R2)–CAT	−108 rOC	WT (2) reverse	642 ± 8.9
WT(2)–CAT	−108 rOC	WT (2) correct	670 ± 33
C1–CAT	−108 rOC	C1 (1) correct	370 ± 6
CM1–CAT	−108 rOC	CM1 (1) correct	80 ± 3.9
pBLCAT2	TK	none	100 ± 6.8
WT–tk–CAT	TK	WT (1) correct	331 ± 7
WT(2)–tk–CAT	TK	WT (2) correct	427 ± 9
WT(R2)–tk–CAT	TK	WT (2) reverse	453 ± 3
CM1–tk-CAT	TK	CM1 (1) correct	98 ± 0.2

Single and multiple copies of wild-type and mutated oligonucleotides corresponding to −151 to −124 nt (see Table 1) were fused upstream of the homologous (−108CAT) and heterologous (pBLCAT2) promoter–CAT constructs in either orientation and transiently transfected into ROS 17/2.8 cells. CAT activity relative to −108CAT and pBLCAT2 controls are indicated and represent mean ± SD of 3–8 experiments, each done in triplicate.

Overexpression of AML-1 Stimulates rOC Gene Expression in Osteoblastic Cells. To examine whether the AML-1 protein is capable of stimulating rOC gene expression by transactivating through the OC Box II sequence, we cotransfected the transactivating isoform, AML-1B (20), in osteoblastic ROS 17/ 2.8 cells with WT-CAT, CM1-CAT, or −108CAT (control). Results (Table 4) demonstrated a 5–7 fold stimulation of OC promoter activity in the presence of WT-CAT construct that contains the intact AML-1-binding motif, whereas the construct containing the mutant oligomer (CM1-CAT) expressed at the same level as the control, −108CAT.

Overexpressed AML-1 Transactivates rOC Gene Expression in Nonosseous Cells. To test the capability of the transactivating form of AML-1 to mediate transcription of this bone-specific promoter in nonosseous cells, we overexpressed AML-1B in the human fibroblastic IMR-90 cell line that does not form the OBSC (see Fig. 1). OC gene expression is absent in this cell line as evidenced by transient transfection results using several promoter deletion-CAT constructs (Fig. 5). However, coexpression of AML-1B with WT-CAT containing the intact AML-1-binding site, resulted in transactivation of the rOC gene in these cells, while the mutant construct

Table 4. Overexpression of AML-1 in ROS 17/2.8 cells

Construct	Overexpressed plasmid	Relative CAT activity
−108CAT	CMV (control)	100 ± 4.8
−108CAT	AML-1B	110 ± 5.1
WT–CAT	CMV (control)	348 ± 22
WT–CAT	AML-1B	2056 ± 190
CM1–CAT	CMV (control)	118 ± 1.1
CM1–CAT	AML-1B	120 ± 0.9

ROS 17/2.8 cells were transiently transfected with 750 ng of AML-1B overexpression plasmid or the vector control and 7 μg of promoter–CAT construct containing wild-type or mutated OC Box II. CAT activity relative to −108CAT (insertless control) cotransfected with CMV (vector) are shown. Data represent mean ± SD of 3–6 experiments, each done in triplicate.

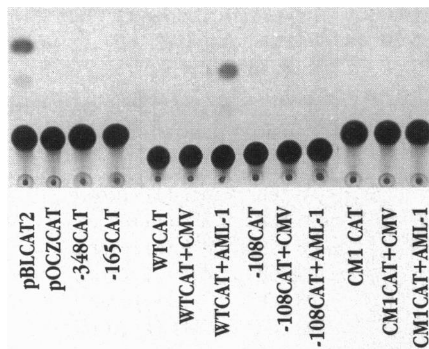

FIG. 5. Overexpression of AML-1 in human fibroblastic cell transactivates the rOC gene through the AML-1-binding site. Representative CAT assay of IMR-90 fibroblastic cells transiently transfected with 10 μg of pBLCAT2 (positive control) and rOC promoter deletion constructs (pOCZCAT, −348CAT, −165CAT) without overexpression of AML-1 are shown on the left. Ten micrograms of WTCAT (WT), CM1CAT (mutant), or −108CAT (control) reporter plasmids were cotransfected with 1 μg of AML-1B overexpression plasmid or the vector control CMV as indicated.

(CM1-CAT) and the control (−108CAT) were unaffected by overexpressed AML-1B. These results indicate that the presence of a transactivating form of AML-1 can also activate rOC gene expression in nonosseous cells through the AML-1-binding motif contained in OC Box II.

DISCUSSION

We have identified an AML-1-binding motif (OC Box II) in the proximal OC promoter that is critical for expression of the gene and binds an osteoblast-specific complex, OBSC, which contains AML-1 and CBFβ. Since the AML-1 family of transcription factors consists of several subtypes that act in a cell-type and tissue-specific manner, we propose that an osteoblast-specific member of this family is involved in formation of the OBSC. Recently, an osteoblast-specific promoter element, OSE2, showing similar transcriptional stimulation of the murine OC gene was reported. The element is homologous to OC Box II and is also present in the human OC promoter (5). Our demonstration of functional stimulatory activity by AML-1/CBF related factors at this site indicates a significant role for these proteins in the regulation of all three OC genes. The presence of the OBSC may be essential for maintenance of a threshold level of expression in the basal promoter of the OC genes. Notably, OC Box II is contiguous to a TGFβ responsive element in the rat OC gene (32). Most of the previous studies on AML-1 related proteins were restricted to cells of hematopoietic lineage; our results indicate for the first time that these AML-related transactivating factors are present and functional in cells of osteoblastic lineage as well.

Our antibody supershift studies indicate that AML-1 and CBFβ are present in osteoblast-derived cells regardless of whether they produce OC (ROS 17/2.8 versus Saos-2). The inability to stimulate or activate OC production in Saos-2, ROS 25/1, or other osteoblastic cell lines may be attributed to modifications of the AML-1 proteins, the presence of a repressor, or the absence of another required factor in these cell lines. Our overexpression studies indicate that in the presence of sufficient cellular levels of the transactivating form of AML-1, IMR-90 cells can activate rOC gene transcription and overcome cell-type and tissue-restricted expression.

Earlier studies suggest that enhancer elements may increase the accessibility of chromatin to transcription factors. The insertion of polyomavirus enhancer into the hypoxanthine phosphoribosyltransferase gene mediates a bidirectional gradient of chromatin accessibility and DNase I hypersensitivity that centers around the enhancer and is lost with the removal of the insert (37). Studies on chromatin structure of the rOC gene show that OC Box II maps to a DNase I hypersensitive region (−170 to −70) of the OC promoter (38). The OC Box II sequence may therefore help increase chromatin accessibility and facilitate the binding of the osteoblast-specific factor(s) at this site. Direct correlation between chromatin structure of the OC Box II domain and basal transcriptional control may be established through further investigations.

In conclusion, the mechanisms by which expression of the rOC gene is controlled in osteoblastic cells are further elucidated by our findings. On the basis of our results, we propose that an osteoblast-specific AML-1 related protein is involved in formation of OBSC and functions as a cell-type restricted transcriptional activator. Further studies using this osteoblast-specific factor are required to establish its involvement in the control of expression of other osteoblast-specific genes.

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