A PEBP2α/AML-1-related Factor Increases Osteocalcin Promoter Activity through Its Binding to an Osteoblast-specific cis-Acting Element

Valérie Geoffroyt, Patricia Ducyt and Gérard Karsenty

From the Department of Molecular Genetics, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

To identify osteoblast-specific cis-acting elements and trans-acting factors, we initiated an analysis of the promoter of a mouse osteocalcin gene, an osteoblast-specific gene. In this promoter, we identified two osteoblast-specific cis-acting elements (Ducy, P. and Karsenty, G. (1995) Mol. Cell. Biol. 15, 1858-1869). The sequence of one of these elements, OSE2, is identical to the DNA-binding site of the PEBP2α/AML-1 transcription factors, the mammalian homologues of the Drosophila Runt protein. Here we show, using nuclear extracts, recombinant protein, and a specific antisera against AML-1, that not only does OSE2 bind to OSE2, but that one member of this family, AML-1B, binds specifically to OSE2 and is immunologically related to OSF2, the factor present in osteoblast nuclear extracts that binds to OSE2. By DNA cotransfection experiments, we also demonstrate that AML-1B can increase the activity of a short osteocalcin promoter through its binding to OSE2. Lastly, the different mobilities of osteoblast nuclear extract-DNA complexes compared with T-cell nuclear extract-DNA complexes, along with the inability of OSF2 to be up-regulated by retinoic acid, unlike the other PEBP2α factors, suggest that OSF2 is a new member of this family of transcription factors. Thus, this study demonstrates that AML-1B can increase gene expression of an osteoblast-specific gene through its binding to an osteoblast-specific cis-acting element and presents evidence that OSF2 is a member of the PEBP2α/AML-1 family of transcription factors.

To date no osteoblast-specific transcription factor has been identified. In an attempt to identify such factors we initiated studies of the cis-acting elements and trans-acting factors that control the expression of the osteocalcin genes in osteoblasts (1). Such studies are crucial for a better comprehension of the molecular mechanisms governing osteoblast differentiation. These studies should also increase our understanding of how, in a number of pathological situations, the synthesis of osteocalcin is altered. These include Paget’s disease, secondary hyperparathyroidism, and some forms of osteoporosis (for review see Ref. 2). We speculate that in these situations the alterations in the expression of the osteocalcin genes are mediated by transcriptional mechanisms.

We previously identified and characterized two osteoblast-specific cis-acting elements in the promoter of mouse osteocalcin gene 2 (mOG2) (1). In DNA transfection experiments, these two elements, called OSE1 and OSE2, activate transcription in osteoblastic cell lines only. OSE1, the most proximal element, binds a factor present in nuclear extracts of mouse primary osteoblasts before they are able to mineralize a matrix but absent from nuclear extracts of either mineralizing primary osteoblasts or other cell lines and mouse tissues tested. This finding suggests that the factor binding to OSE1, whose characterization is underway, could be a stage-specific, osteoblast-specific transcription factor.

OSE2, the second osteoblast-specific cis-acting element we have characterized, binds a factor present in nuclear extracts of primary osteoblasts, regardless of their stage of differentiation but absent in nuclear extracts of any other cell lines or mouse tissues tested. In this report, we have named this factor osteoblast-specific factor 2 or OSF2. A systematic site-specific mutagenesis of OSE2 enabled us to define a core sequence of 8 bp. A single base pair substitution mutation of any of these 8 bp either abolishes or greatly reduces the binding of OSF2 to OSE2. When we compared the OSE2 sequence with the binding sites of known transcription factors (3), we found an identity between it and the DNA-binding site of AML-1 (4, 5), a human transcription factor, and of PEBP2α, the mouse counterpart of AML-1 (6–9).

AML-1 belongs to a family of transcription factors conserved between Drosophila and human (7). The first gene to be identified in this family was the Drosophila segmentation gene runt (10). The mouse homologues of runt include PEBP2αA (6) and PEBP2αB (7). To date, three human genes, AML-1A (4), AML-1B (5), and PEBP2αC (9), have been identified. The mouse and human genes have in common with their Drosophila counterpart a conserved domain called the runt domain, which encodes a 128-amino acid peptide. This domain is responsible for DNA binding and heterodimerization (7, 11). The PEBP2α/AML-1 factors identified and studied has all been implicated in myeloid cell-specific gene expression (5, 7, 12–14).

In this study, we conducted a biochemical, immunological, and functional characterization of OSF2, the factor present in osteoblast nuclear extracts and binding to OSE2. We present evidence that OSF2 is immunologically related to the PEBP2α/AML-1 transcription factors. Furthermore, we show by DNA
Osteoblast-specific Gene Expression, PEBP2α/AML-1 Factors

**MATERIALS AND METHODS**

Cell Culture and DNA Transfection—Mouse F9 teratocarcinoma cells were cultured in Eagle's minimal essential medium/10% fetal bovine serum. The ROS 17/2.8 osteosarcoma cell line and the mouse primary osteoblasts were cultured as described previously (1). The human T-lymphoblastic cell lines Molt 4 and Jurkat were maintained in RPMI 1640 medium supplemented with 10% calf serum. The day before transfection, F9 cells were plated on 10-cm dishes at a density of 5 × 10^5 cells/dish. Cells were transfected by the calcium phosphate coprecipitation method (15), using 5 μg of reporter plasmid, 5 μg of expression vector, and 2 μg of pRSV/β-gal. After transfection, the cells were washed twice with phosphate-buffered saline (150 mM NaCl, 10 mM sodium phosphate (pH 7.2)) and then incubated in regular medium for 24 h. Cells were harvested by scraping them into 0.3 ml of 0.25M Tris-HCl (pH 7.8) and lysed by three cycles of freezing-thawing. β-Galactosidase activities present in each lysate, measured by a colorimetric enzyme assay using resorufin β-β-galactopyranoside (Boehringer Mannheim) as a substrate, were used to normalize the transfection efficiency between experiments. Luciferase activities were assayed by using a Monolight 2010 luminometer (Analytical Luminescence Laboratory) and d-luciferin substrate (Analytical Luminescence Laboratory) in 100 mM Tris-HCl (pH 7.8), 5 mM ATP, 15 mM MgSO4, and 1 mM dithiothreitol. To induce their differentiation, the F9 cells were plated and treated with 1 μM retinoic acid (RA) (Sigma) for 5 days, as described previously (16). On the second day, the medium was changed, and RA was added again. More than 95% of the cells became differentiated as judged microscopically on the fifth day of incubation. ROS 17/2.8 cells were treated with RA in the same conditions and for the same time.

In Vitro Transcription and Translation—The AML-1B plasmid DNA was transcribed and translated using the Tnt kit (Promega), and unlabeled or 35S-labeled methionine for 90 min at 30 °C in parallel. 35S-labeled proteins were analyzed by SDS-polyacrylamide gel electrophoresis, and unlabeled products were used in a gel retardation assay. The AML-1B plasmid was kindly provided by Dr. S. Hiebert (St. Jude Children's Research Hospital, Memphis, TN).

DNA Constructions—Details of the construction of the reporter plasmids p34-luc, p60OSE2-luc, p60OSE1-luc, and p60CE1-luc, vectors containing, respectively, 6 copies of the wild-type OSE 2 oligonucleotide, 6 copies of the mutant OSE 2 oligonucleotide, or 6 copies of the wild-type OSE 1 oligonucleotide in front of the −34/−13 MGO2 promoter-luciferase (luc) chimeric gene, have been reported (1). The construction of expression vectors containing the AML-1B cDNA cloned, in the correct or opposite orientation, downstream of the CMV5 promoter has been reported (5). These expression vectors were a generous gift of Dr. S. Hiebert. The construction of the expression vector containing the c-Myb cDNA cloned downstream of the DHFR promoter has been reported earlier (17), this plasmid was a generous gift of Dr. B. Calabretta (Jefferson Cancer Institute, Thomas Jefferson University, Philadelphia, PA).

Nuclear Extract Preparation and DNA Binding Assays—Nuclear extracts were prepared according to Dignam et al. (18) and Schreier et al. (19). Buffers for the preparation of nuclear extracts contained 0.5 mM dithiothreitol and the protease inhibitors 0.5 mM phenylmethylsulfonyl fluoride and 10 μg/ml of leupeptin and pepstatin. For the gel retardation assays (GRA), labeled double-stranded oligonucleotides were prepared as described previously (1). DNA binding and electrophoresis conditions were also as described previously (1, 5). The oligonucleotides used in this study are presented in Table I. For competition experiments, the indicated amount of double-stranded unlabeled oligonucleotide was added to the binding reaction with the other components. For experiments using anti-AML-1B antiserum, which was raised against a 17-amino acid N-terminal peptide of AML-1 (4), the indicated amount of double-stranded unlabeled oligonucleotide was added to the binding reaction with the other components. When we used as a probe in GRA and OSE2 binding site were used in the study in the same conditions and for the same time.

**RESULTS**

The OSE2 Sequence and PEBP2α/AML-1 Binding Sites Are Similar—A comparison of the OSE2 sequence 5′ AACCACA 3′ with the binding site of known transcription factors (3) showed that the most homologous binding sites were those for AML-1 and PEBP2α, a group of transcription factors conserved between mouse and human that recognize the DNA sequence 5′PuCCPuAC3′ (Fig. 1). Given this sequence homology, we tested whether oligonucleotides containing either the OSE2 sequence or the Moloney murine leukemia virus PEBP2α binding site (20) would bind similar factors present in nuclear extracts of ROS 17/2.8 cells.

When we used the OSE2 oligonucleotide as a probe in GRA and ROS 17/2.8 nuclear extracts as a source of proteins, we observed a protein-DNA complex already described (Fig. 2, lane 1) (1). The formation of this complex was inhibited by a 50-fold molar excess of the wild-type OSE2 oligonucleotide or the wild-type PEBP2α oligonucleotide (Fig. 2, lanes 2-7). This complex was unaffected by 100-fold of a mutant OSE2 oligonucleotide containing a 2-bp substitution mutation or of a mutant PEBP2α oligonucleotide containing the same 2-bp substitution mutation (Fig. 2, lanes 8 and 9). When we used as a probe in

| Name                  | Sequence                      | Reference |
|-----------------------|-------------------------------|-----------|
| OSE2 wild-type        | GATCCGCTCAATCACACCAAGACCA    | 1         |
| OSE2 mutant           | GGGCTTATGCTGTGCTGCTGCTAG      | 1         |
| PEBP2α wild-type      | GGGGATATCTGTGGTAAGCA          | 20        |
| PEBP2α mutant         | CTTATAGACCACTTGGTGG           | This study |
| Sp1                   | AGGGCCCAGGGATTCGGCGGG         | 21        |

**Table I**

| Oligonucleotides used in this study | Name     | Sequence                      | Reference |
|-----------------------------------|----------|-------------------------------|-----------|
|                                   | OSE2     | GATCCGCTCAATCACACCAAGACCA    | 1         |
|                                   | OSE2     | GGGCTTATGCTGTGCTGCTGCTAG      | 1         |
|                                   | PEBP2α   | GGGGATATCTGTGGTAAGCA          | 20        |
|                                   | PEBP2α   | CTTATAGACCACTTGGTGG           | This study |
|                                   | Sp1      | AGGGCCCAGGGATTCGGCGGG         | 21        |
GRA the PEBP2α oligonucleotide and incubated it with ROS 17/2.8 nuclear extracts, we detected a protein-DNA complex that had the same mobility pattern as the one formed upon incubation of these nuclear extracts with the labeled OSE2 oligonucleotide (Fig. 2, compare lanes 1 and 10). Formation of this protein-DNA complex was inhibited by a 50–100-fold molar excess of the wild-type OSE2 oligonucleotide or the wild-type PEBP2α oligonucleotide (Fig. 2, lanes 11–16). A large excess of mutant OSE2 oligonucleotide or mutant PEBP2α oligonucleotide containing the 2-bp substitution mutation failed to inhibit this binding (Fig. 2, lanes 17 and 18). In summary, the equal mobility of the complexes and the results of the DNA competition experiments indicate that OSF2, the factor present in osteoblast nuclear extracts and binding to OSE2, is related to the PEBP2α/AML-1 proteins. The fact that in DNA competition experiments the same molar excess of the PEBP2α oligonucleotide and the OSE2 oligonucleotide was necessary to abolish binding of OSF2 to either probe suggests that OSF2 has a similar affinity for the PEBP2α binding site and OSE2.

Recombinant AML-1B Protein Binds Specifically to OSE2—We then used ivt AML-1B protein to perform GRA. The ivt Protein had been shown to bind to the PEBP2α binding site and to the AML-1B binding site (5). The ivt AML-1B protein bound to the labeled OSE2 oligonucleotide; this binding was specific because it could be abolished by a 200-fold molar excess of the wild-type OSE2 oligonucleotide but not by a 400-fold molar excess of the mutant OSE2 oligonucleotide (Fig. 3A, lanes 1–4). As a control, we performed the same experiment using the wild-type PEBP2α oligonucleotide as a probe and showed that ivt AML-1B bound specifically to this oligonucleotide (Fig. 3A, lanes 5–11). The ivt AML-1B-DNA complex always had a slightly slower mobility when we used the PEBP2α oligonucleotide than when we used the OSE2 oligonucleotide. We then used an antiserum directed against the N-terminal part of AML-1B in GRA. As shown in Fig. 3B (lane 3), this antibody inhibited binding of ivt AML-1B protein to the OSE2 oligonucleotide. To show the specificity of this interaction, we performed two control experiments. First, when added during the binding reaction, the peptide against which this antiserum was raised prevented the abolition of binding (Fig. 3B, lane 4). Second, a purified antibody against an unrelated transcription factor, c-Myb, had no effect on the binding of the ivt AML-1B protein to the labeled OSE2 oligonucleotide (Fig. 3B, lane 5). As previously reported, the binding of ivt AML-1B to the PEBP2α oligonucleotide was abolished specifically by the anti-AML-1B antiserum but not by the unrelated anti-c-Myb antibody (Fig. 3B, lanes 6–10).

We then asked whether the anti-AML-1B antiserum could alter the binding of ROS 17/2.8 or mouse primary osteoblast nuclear extracts to the labeled OSE2 oligonucleotide. As shown in Fig. 4, the addition of increasing amounts of anti-AML-1B antiserum substantially decreased or abolished the binding of either ROS 17/2.8 nuclear extracts or mouse primary osteoblast nuclear extracts to the OSE2 oligonucleotide (Fig. 4, lanes 2–5 and 11–14). As was the case for the ivt AML-1B protein, this decreased binding was inhibited by the addition during the binding reaction of the AML-1 antigenic peptide against which the antiserum was raised (Fig. 4, lanes 8 and 17). This inhibition of binding was not observed when we used a 10-fold higher amount of an anti-c-Myb antibody as a control (Fig. 4, lanes 9 and 18). We chose an antibody against c-Myb because this is also a myeloid-specific transcription factor that binds to a sequence related but not identical to the OSE2 sequence (17). In summary, the specific binding of the ivt AML-1B protein to the OSE2 oligonucleotide, as well as the abolition of binding of osteoblast nuclear extracts to the OSE2 oligonucleotide by the addition of the anti-AML-1B antiserum in the binding reaction, indicates that a PEBP2α/AML-1-related protein is present in osteoblast nuclear extracts and binds specifically to OSE2. As stated above we named this PEBP2α/AML-1-related factor OSF2. We next asked whether we could distinguish OSF2 from the other PEBP2α factors.

OSF2, the OSE2 Binding Factor, and the Factor Present in Lymphocytes Nuclear Extracts Have Different Mobilities in GRA—We showed earlier that OSF2 was present only in nuclear extracts of primary osteoblasts or osteoblastic cell lines (1). This initial analysis included nuclear extracts of a mouse B-cell line as one of our negative controls but did not include nuclear extracts of T-cell lines or other hematopoietic cell lines known to contain PEBP2α factors. Given the results presented above, we performed a GRA using nuclear extracts of mouse primary osteoblasts, ROS 17/2.8 osteoblastic cells, the 3 194 mouse B-cells, and two different human T-cell lines known to contain PEBP2α factors, the Jurkat and the Molt 4 cell lines (4). The mobility of the protein-DNA complexes formed upon incubation of osteoblast nuclear extracts with the OSE2 oligonucleotide or the PEBP2α oligonucleotide were different from the mobility of the protein-DNA complexes formed upon incubation of human T-cell nuclear extracts with the same oligonucleotides (Fig. 5A, lanes 1–2, 4–5, 6–7, and 9–10). Interestingly, the protein-DNA complex formed upon incubation of mouse
used we showed that intact Sp1 binding activity was present (Fig. 5C).

The Synthesis of OSF2 Is Not Inducible by Retinoic Acid Treatment of F9 or ROS 17/2.8 Cells—The PEBP2α factors are not detectable in nuclear extracts of untreated F9 mouse teratocarcinoma cells but are present in nuclear extracts of F9 cells differentiated by a 5-day treatment with RA (16). We took advantage of this feature of the PEBP2α factors to determine whether the mobility of differentiated F9 nuclear extracts-DNA complexes was identical to the mobility of osteoblast nuclear extracts-DNA complexes in GRA. We generated nuclear extracts of F9 cells either untreated or treated with RA for 5 days and used them in a GRA. As shown previously (1), no OSE2 binding activity was present in nuclear extracts of untreated F9 cells (Fig. 5B, lane 2). When we used nuclear extracts of RA-treated cells and a labeled OSE2 oligonucleotide as a probe in GRA, we observed a complex of weak intensity that had a mobility similar to the one formed upon incubation of lymphocytes nuclear extracts with the OSE2 oligonucleotide (compare Fig. 5B, lane 3, to Fig. 5A, lanes 4 and 5) but clearly different from the one formed upon incubation of osteoblast nuclear extracts with this probe (Fig. 5B, lane 1). As described previously (16), identical results were obtained when we used the labeled PEBP2α oligonucleotide as a probe (data not shown). We also used nuclear extracts of ROS 17/2.8 osteoblastic cells that were either untreated and treated with RA under the same conditions. As shown in Fig. 5B (lanes 4 and 5), treatment of ROS 17/2.8 with RA did not significantly increase the intensity of the already existing protein-DNA complex, nor did it generate a new protein-DNA complex, even after a longer exposure (data not shown). In summary, this experiment confirms that the synthesis of PEBP2α factors can be induced in F9 cells treated for 5 days with RA, but it also indicates that the synthesis of OSF2 is not up-regulated by RA. Thus this experiment suggests that OSF2 may be a new member of the PEBP2α/AML-1 family of transcription factors.

AML-1B Increases mOG2 Promoter Activity through Its Binding to OSE2—To determine the functional relevance of the binding of AML-1B to OSE2, we performed DNA cotransfection experiments. For this assay, we chose the mouse cell line F9, because it does not express either osteocalcin or PEBP2α and so provides a null background. We used three different expression vectors, one bearing the AML-1B cDNA in the correct orientation, one bearing the AML-1B cDNA in the opposite orientation (both driven by the CMV promoter), and one bearing the c-Myc cDNA in the correct orientation driven by the DHFR promoter (Fig. 6A). The reporter plasmids we used contained the coding region of the luciferase (luc) gene driven by a −34/+13 mOG2 promoter fragment that has no transcriptional activity (1). Upstream of this promoter, we cloned six copies of a wild-type OSE2 oligonucleotide (p6OSE2-luc), six copies of a mutant OSE2 oligonucleotide (p6OSE2 m-luc), or six copies of a wild-type OSE1 oligonucleotide, the osteoblast-specific cis-acting element present in the mOG2 promoter (pOSE1-luc) (1) (Fig. 6A). As shown in Fig. 6B, very low levels of luc activity were detected in cells transfected with the minimal −34/+13 mOG2 promoter or p6OSE2-luc alone, whereas cotransfection of the p6OSE2-luc reporter plasmid along with the AML-1B cDNA cloned in the correct orientation stimulated luc expression 200-fold. To demonstrate the specificity of this effect, we performed several control experiments. First, no stimulation occurred when we used the expression vector containing AML-1B cDNA in the opposite orientation. Second, the activity of the p6OSE2 m-luc reporter plasmid was unaffected by cotransfection with CMV-AML-1B fusion gene. Third, the activity of the pOSE1-luc reporter plasmid was not affected by
cotransfection with CMV-AML-1B fusion gene. Lastly, an expression vector containing the cDNA and coding another myeloid-specific transcription factor, c-Myb, did not induce any increase of the activity of p6OSE2-luc. Thus, these results establish that AML-1B is capable of increasing the activity of a short mOG2 promoter through its binding to OSE2 and that this effect is specific.

DISCUSSION

The results presented here show that a member of the PEBP2a/AML-1 family of transcription factors can activate mOG2 expression through its binding to OSE2, an osteoblast-specific cis-acting element (1). This conclusion is based on several experimental arguments. First, OSE2 contains a sequence identical to the binding site of PEBP2a/AML-1 factors. Second, an ivt AML-1B binds specifically to an oligonucleotide containing the OSE2 sequence. Third, an antibody recognizing several members of the PEBP2a/AML-1 family of transcription factors greatly decreases or abolishes the binding of osteoblast nuclear extracts to the OSE2 oligonucleotide. Finally, in DNA cotransfection experiments an expression vector containing AML-1B cDNA, the human homologue of the murine PEBP2aB, increases activity of a short osteocalcin promoter through multimers of OSE2.

Several lines of evidence suggest that the binding of recombinant AML-1B to OSE2 and the activation of the mOG2 promoter by this factor are specific. An antibody directed against another transcription factor did not alter binding of ROS17/2.8 nuclear extracts to the OSE2 oligonucleotide. When we used other ivt transcription factors as control in GRA, they did not bind to the OSE2 oligonucleotide (data not shown). Most importantly, in DNA cotransfection experiments in F9 cells, which express neither the osteocalcin genes nor the PEBP2a genes, AML-1B, dramatically increased (200-fold) the expression of a minimum mOG2 promoter bearing six copies of the wild-type OSE2 oligonucleotide but had no effect on a promoter bearing six copies of the mutant OSE2 oligonucleotide or six copies of the wild-type OSE1 oligonucleotide, the other osteoblast-specific cis-acting element present in the mOG2 pro-
A

Expression vectors

pCMV-AML-1B

pCMV-β1-TIV

pMMU-DHFR

p6OSF2-luc

p6OSF2m-luc

p6OSE1-luc

pCMV-AML-1B

pCMV-β1-TIV

pMMU-DHFR

p6OSF2-luc

p6OSF2m-luc

p6OSE1-luc

pCMV-AML-1B

A. Expression vectors and reporter plasmids. Expression vectors, pCMV-AML-1B sense: the AML-1B cDNA was dosed downstream of the CMV promoter in the correct orientation; pCMV-AML-1B antisense: the AML-1B cDNA was dosed downstream of the CMV promoter in the opposite orientation; pβ1-TIV DHFR; the c-Myc cDNA was dosed downstream of the DHFR promoter in the correct orientation. Reporter plasmids, all the reporter plasmids contain the −34/+13 mOG2 promoter-luciferase fusion gene. p6OSF2-luc contains six copies of the wild-type OSE2 oligonucleotide cloned upstream of the −34/+13 mOG2 promoter. p6OSF2 m-luc contains six copies of the mutant OSE2 oligonucleotide cloned upstream of the −34/+13 mOG2 promoter. p6OSE1-luc contains six copies of the wild-type OSF2 oligonucleotide cloned upstream of the −34/+13 mOG2 promoter. B. F9 cells were transiently transfected with 5 μg of the indicated reporter plasmid in the absence (−) or presence (+) of the indicated expression vector. Values are expressed relatively to the basal activity of p0OSE2-luc, which was set at 1. The data represent results 3–6 independent transfection experiments.

B

Reporter
plasmid

Expression
vector

p34-luc

pCMV-AML-1B

pCMV-AML-1B

pβ1-TIV

pMMU-DHFR

p6OSF2-luc

pCMV-AML-1B

pCMV-AML-1B

pβ1-TIV

pMMU-DHFR

p6OSE1-luc

pCMV-AML-1B

Fold induction

50

100

150

200

FIG. 6. AML-1B activates transcription of the osteocalcin promoter in F9 cells through OSE2. A, representation of the expression vectors and reporter plasmids. Expression vectors, pCMV-AML-1B sense: the AML-1B cDNA was dosed downstream of the CMV promoter in the correct orientation; pCMV-AML-1B antisense: the AML-1B cDNA was dosed downstream of the CMV promoter in the opposite orientation; pβ1-TIV DHFR: the c-Myc cDNA was dosed downstream of the DHFR promoter in the correct orientation. Reporter plasmids, all the reporter plasmids contain the −34/+13 mOG2 promoter-luciferase fusion gene. p6OSF2-luc contains six copies of the wild-type OSE2 oligonucleotide cloned upstream of the −34/+13 mOG2 promoter. p6OSF2 m-luc contains six copies of the mutant OSE2 oligonucleotide cloned upstream of the −34/+13 mOG2 promoter. p6OSE1-luc contains six copies of the wild-type OSF2 oligonucleotide cloned upstream of the −34/+13 mOG2 promoter. B. F9 cells were transiently transfected with 5 μg of the indicated reporter plasmid in the absence (−) or presence (+) of the indicated expression vector. Values are expressed relatively to the basal activity of p0OSE2-luc, which was set at 1. The data represent results 3–6 independent transfection experiments.

The murine transcription factor PEBP2 was originally identified as a factor binding to the polyomavirus core enhancer (23). It is composed of two distinct peptides, PEBP2α and PEBP2β (11). To date two distinct PEBP2α factors, encoded by two distinct genes, have been identified: PEBP2αA, and PEBP2αB. The two PEBP2α factors have in common a DNA-binding domain highly homologous to the DNA-binding domain of runt, a segmentation gene in Drosophila. This runt domain also allows the PEBP2α proteins to dimerize with PEBP2β, a ubiquitously expressed nuclear factor that itself does not bind to DNA but does increase the affinity of PEBP2α factors for their binding sites (11). The two genes PEBP2αA and PEBP2αB have a different pattern of expression: PEBP2αA is expressed in T-cells (7), PEBP2αB is expressed in lung and pre-B-cells in addition to the T-cells (8, 9). Three human homologues of PEBP2α, AML-1A, and AML-1B and PEBP2αC have been characterized recently. AML-1A was identified as a breakpoint on chromosome 21 (t(8;21)) present in a chronic myeloid leukemia (24), AML-1B was cloned in a B-lymphocyte cDNA library (5), and PEBP2αC is largely distributed (9).

The most important question raised by the present study is whether OSF2, the factor present in osteoblast nuclear extracts and binding to the OSE2 oligonucleotide, is an already known PEBP2α factor or a new but biochemically and immunologically related factor. In this study, we showed that the protein-DNA complexes formed upon incubation of either osteoblast nuclear extracts or T-cell nuclear extracts, with OSE2 oligonucleotide have a different mobility in GRA. We obtained identical results when using PEBP2α oligonucleotide as a probe. A recent study of PEBP2αA and PEBP2αB patterns of expression during mouse development did not mention that either of these two genes is expressed in developing skeleton (25). This report also mentioned that PEBP2αA transcripts were abundant in lung, a tissue that has no detectable OSE2 binding activity (1). Lastly, the disruption of the human homologue of PEBP2αB, AML-1A, is associated with myeloid leukemia but not with skeletal abnormalities (24). These three indirect lines of evidence suggest that OSF2, the factor present in osteoblast nuclear extracts and binding to OSE2, is different from the previously described PEBP2α factors. More compelling although still indirect evidence suggesting that OSF2 is a previously undescribed PEBP2α factor comes from our experiments using nuclear extracts of RA-treated F9 or ROS 17/2.8 cells. This treatment increased only the synthesis of PEBP2α factors that form protein-DNA complexes of a faster mobility when incubated with the OSE2 oligonucleotide, compared with the protein-DNA complex formed upon incubation of osteoblast nuclear extracts with this oligonucleotide. The synthesis of OSF2 itself could not be up-regulated by RA using the same treatment conditions.

We are fully aware that this experimental evidence is indirect; a definite proof of the identity of the PEBP2α-related factor binding to OSE2 will have to await the cloning and characterization of OSF2 and PEBP2α cDNAs from osteoblastic cDNA libraries. If OSF2 is one of the three described PEBP2α factors, the mechanisms by which it affects osteoblast differentiation alone or in association with other factors will have to be investigated in great detail because the osteocalcin genes are not expressed in lymphoid tissues.

Besides the osteoblast-specific cis-acting elements we identified in the mOG2 promoter, several regions of various genes expressed in osteoblasts have been recognized recently as containing osteoblast-specific cis-acting elements. In the osteocalcin promoter a binding site for the homeodomain containing proteins MSX1 and MSX2 has been identified (26), although the role of the MSX proteins in regulating osteocalcin gene expression is unclear (27). Using a transgenic mouse approach Pavlin et al. (28) have identified a region of the rat α1(I) collagen promoter required for osteoblast-specific expression of a reporter gene in vivo. This region is centered by a typical c-is matured (I) pattern of expression, whereas the osteocalcin genes are late markers of osteoblast differentiation. Given these different temporal patterns of expression, it is not surprising that different osteoblast-specific cis-acting elements and trans-acting factors regulate the expression of the osteocalcin genes and the type I collagen genes. 

In summary, we have presented evidence that AML-1B binds specifically to OSE2, an osteoblast-specific cis-acting element, and increases the transcriptional activity of a short mOG2.
promoter through its binding to OSE2. This study also shows that OSF2, the factor present in osteoblast nuclear extracts and binding to OSE2, has different characteristics than the already described PEBP2α factors. The precise identity of OSF2 and its relationship to the known PEBP2α factors, as well as the molecular basis for its activation of the osteocalcin gene expression, are currently under investigation.

Acknowledgments—We are very grateful to Drs. Meyers and Hiebert (St. Jude Children’s Research Hospital, Memphis, TN), who generously provided us with reagents that made this study possible. We also thank Dr. B. Calabretta (Jefferson Cancer Institute, Thomas Jefferson University, Philadelphia, PA) for the generous gift of the expression vector pMbMI/DHFR.

REFERENCES

1. Ducy, P., and Karsenty, G. (1995) Mol. Cell. Biol. 15, 1858–1869
2. Hauschka, P. V., Lian, J. B., Cole, D. E. C., and Gundberg, C. M. (1989) Physiol. Rev. 69, 990–1047
3. Boulikas, T. (1994) Crit. Rev. Eukaryotic Gene Expression 4, 117–321
4. Meyers, S., Downing, J. R., and Hiebert, S. W. (1993) Mol. Cell. Biol. 13, 6336–6345
5. Meyers, S., Lenny, N., and Hiebert, S. W. (1995) Mol. Cell. Biol. 15, 1874–1892
6. Bae, S.-C., Yamaguchi-Iwai, Y., Ogawa, E., Maruyama, M., Inuzuka, M., Kagoshima, H., Shigesada, K., Satake, M., and Ito, Y. (1992) Oncogene 8, 809–814
7. Ogawa, E., Maruyama, M., Kagoshima, H., Inuzuka, M., Lu, J., Satake, M., Shigesada, K., and Ito, Y. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 6859–6863
8. Bae, S.-C., Ogawa, E., Maruyama, M., Oka, H., Satake, M., Shigesada, K., Jenkens, N. A., Gilbert, D. J., Copeland, N. G., and Ito, Y. (1994) Mol. Cell. Biol. 14, 3242–3252
9. Bae, S.-C., Takahashi, E.-I., Zhang, Y. W., Ogawa, E., Shigesada, K., Namba, Y., Satake, M., and Ito, Y. (1995) Gene (Amst.) 159, 245–248
10. Kania, M. A., Bonner, A. S., Duffy, J. B., and Gergen, J. P. (1990) Gene & Dev. 4, 1701–1713
11. Ogawa, E., Inuzuka, M., Maruyama, M., Satake, M., Naito-Fujimoto, M., Ito, Y., and Shigesada, K. (1993) Virology 194, 314–331
12. Nuchprayoon, I., Meyers, S., Scott, L. M., Suzow, J., Hiebert, S., and Friedman, A. D. (1994) Mol. Cell. Biol. 14, 5558–5568
13. Giese, K., Kingsley, C., Kirshner, J. R., and Grosschedl, R. (1995) Gene & Dev. 9, 995–1008
14. Zhang, D.-E., Fujikawa, K., Hetherington, C. J., Shapiro, L. H., Chen, H.-M., Look, A. T., and Tenen, D. G. (1994) Mol. Cell. Biol. 14, 8035–8040
15. Chen, C., and Okayama, H. (1987) Mol. Cell. Biol. 7, 2745–2752
16. Furukawa, K., Yamaguchi, Y., Ogawa, E., Shigesada, K., Satake, M., and Ito, Y. (1990) Cell Growth & Differ. 1, 139–147
17. Clarke, M. F., Kukowska-Latallo, J. F., Westin, E., Smith, M., and Prochownik, E. V. (1988) Mol. Cell. Biol. 8, 884–892
18. Dignam, J. D., Lebowitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475–1489
19. Schreiber, E., Matthias, P., Muller, M. M., and Schaffner, W. (1989) Nucleic Acids Res. 17, 6419
20. Wang, S., and Spек, N. A. (1992) Mol. Cell. Biol. 12, 89–102
21. Gidoni, D., Dyanan, W. S., and Tjian, R. (1984) Nature 312, 409–413
22. Satake, M., Inuzuka, M., Shigesada, K., Okawa, T., and Ito, Y. (1992) Proc. Natl. Acad. Sci. U.S.A. 90, 6859–6863
23. Kamachi, Y., Ogawa, E., Asano, M., Ishida, S., Murakami, Y., Satake, M., Ito, Y., and Shigesada, K. (1990) J. Virol. 64, 4808–4819
24. Liu, P., Tarle, S. A., Hajra, A., Clayton, D. F., Mariton, P., Freedman, M., Siciliano, M. J., and Collins, F. S. (1993) Science 261, 1041–1044
25. Satake, M., Nomura, S., Yamaguchi-Iwai, Y., Takahama, Y., Hashimoto, Y., Niki, M., Kitamura, Y., and Ito, Y. (1995) Mol. Cell. Biol. 15, 1662–1670
26. Towler, D. A., Bennett, C. D., and Rodan, G. A. (1994) Mol. Endocrinol. 8, 614–624
27. Towler, D. A., Rutledge, S. J., and Rodan, G. A. (1994) Mol. Endocrinol. 8, 1484–1493
28. Pavlin, D., Lichter, A. C., Bedalov, A., Kream, B. E., Harrison, J. R., Thomas, H. F., Gronowicz, G. A., Clark, S. H., Woody, C. L., and Rowe, D. W. (1992) J. Cell. Biol. 116, 227–236
A PEBP2/AML-1-related Factor Increases Osteocalcin Promoter Activity through Its Binding to an Osteoblast-specific cis-Acting Element
Valérie Geoffroy, Patricia Ducy and Gérard Karsenty

J. Biol. Chem. 1995, 270:30973-30979.
doi: 10.1074/jbc.270.52.30973

Access the most updated version of this article at http://www.jbc.org/content/270/52/30973

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 28 references, 17 of which can be accessed free at http://www.jbc.org/content/270/52/30973.full.html#ref-list-1