Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 1027–1031, February 1996
Genetics

Identification of a minimal sequence of the mouse pro-α1(I) collagen promoter that confers high-level osteoblast expression in transgenic mice and that binds a protein selectively present in osteoblasts

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Communicated by Don W. Fawcett, Harvard Medical School, Boston, MA, September 11, 1995

ABSTRACT **Based on our previous transgenic mice results, which strongly suggested that separate cell-specific cis-acting elements of the mouse pro-α1(I) collagen promoter control the activity of the gene in different type I collagen-producing cells, we attempted to delineate a short segment in this promoter that could direct high-level expression selectively in osteoblasts. By generating transgenic mice harboring various fragments of the promoter, we identified a 117-bp segment (−1656 to −1540) that is a minimal sequence able to confer high-level expression of a *lacZ* reporter gene selectively in osteoblasts when cloned upstream of the proximal 220-bp pro-α1(I) promoter. This 220-bp promoter by itself was inactive in transgenic mice and unable to direct osteoblast-specific expression. The 117-bp enhancer segment contained two sequences that appeared to have different functions. The A sequence (−1656 to −1628) was required to obtain expression of the *lacZ* gene in osteoblasts, whereas the C sequence (−1575 to −1540) was essential to obtain consistent and high-level expression of the *lacZ* gene in osteoblasts. Gel shift assays showed that the A sequence bound a nuclear protein present only in osteoblastic cells. A mutation in the A segment that abolished the binding of this osteoblast-specific protein also abolished *lacZ* expression in osteoblasts of transgenic mice.**

Differentiation of mesenchymal cells into osteoblasts is a multistep process that begins around day 13–14 of mouse embryonic development in discrete areas of the primordial skeleton. Concurrently with the differentiation of osteoblasts, synthesis of several extracellular matrix proteins occurs, among which type I collagen is a major constituent. Cytokines, including bone morphogenetic proteins, play an important role in these processes of differentiation (1). Little is known, however, about the molecular mechanisms that control osteoblast differentiation and whether specific transcription factors are involved.

Previous data obtained with transgenic mice harboring various fragments of the mouse pro-α1(I) collagen promoter linked to the *Escherichia coli* β-galactosidase gene strongly suggested that separate cell-specific cis-acting elements control the activity of the gene in different type I collagen-producing cells (2). Results from other laboratories obtained with mice transgenic for the human or the rat pro-α1(I) collagen gene are also consistent with this hypothesis (3–5). In mice, as well as in these two species, the element responsible for high-level expression of the reporter gene in bone appears to be located within the first 2.3 kb of the promoter (2–6). Here we asked whether the postulated "osteoblast-specific" element in the mouse pro-α1(I) collagen gene could be more precisely identified. This would also directly test the hypothesis of a modular organization of separate cis-acting elements. We report the identification of a minimal 117-bp sequence that conferred high-level selective expression of the *lacZ* gene in osteoblasts of transgenic mice and was shown to bind a DNA-binding protein present selectively in osteoblastic cell lines.

MATERIALS AND METHODS

Plasmid Constructions. Two expression vectors were used in this study: placH, which contains the *lacZ* reporter gene, and pluc4, which contains the firefly luciferase gene (2). The constructs contained, upstream of the reporter gene (Fig. 1), (*i*) a *Bgl* II/*Xba* I fragment of the pro-α1(I) collagen gene extending from −220 to +110, (*ii*) various segments of the pro-α1(I) proximal promoter located between −2.3 kb and −0.2 kb, and (*iii*) a 2954-bp "matrix attachment region" of the chicken lysozyme locus (7, 8). The so-called matrix attachment region has been shown to increase the ratio of transgenic mice expressing a reporter gene to those that had integrated the transgene and to allow a more faithful developmental regulation of this reporter gene (8). We showed previously that this DNA segment does not modify the pattern of expression of the *lacZ* and luciferase reporter genes in transgenic mice harboring 2.3 kb of the proximal pro-α1(I) promoter (2). The segments of the pro-α1(I) promoter were obtained by digestion with restriction enzymes and/or by using synthetic double-stranded (ds) oligonucleotides. The −1627 to −1624 4-bp deletion (2300dellacZ) was obtained by digestion with *Pst* I and then with nuclease S1. The −1636 to −1639 4-bp mutation (AATT to GGCC) was introduced by PCR (17-CM1lacZ). The sequences of all ds oligonucleotides and PCR products were verified after cloning. In all but three cases (23-16lacZ, 16-09lacZ, and 2300dellacZ), the segments of the pro-α1(I) promoter were multimerized (four copies) in a head-to-tail orientation before being cloned in the placH expression vector. Multimerization was made possible by introducing a *Bam*HI site at one end of the segment and a *Bgl* II site at the other end.

Generation of Transgenic Mice. Transgenic mice were generated as described (2). Each of the three luciferase constructs (m.p.lucif, 23-16lucif, and 16-09lucif) was coinjected with the corresponding β-galactosidase construct containing the same segments of the pro-α1(I) collagen gene to obtain transgenic mice coexpressing the two reporter genes. The integration of the reporter genes was verified by Southern analysis of either tail DNA or placenta DNA, as described (2). Transgenic mice harboring the luciferase constructs and two transgenic mice harboring the 17-13lacZ construct were used to generate lines. Otherwise, all founder mice were sacrificed at day 15.5 post-conception (p.c.) and stained with 5-bromo-4-chloro-3-indolyl

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Abbreviations: ds, double stranded; p.c., postconception; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside.

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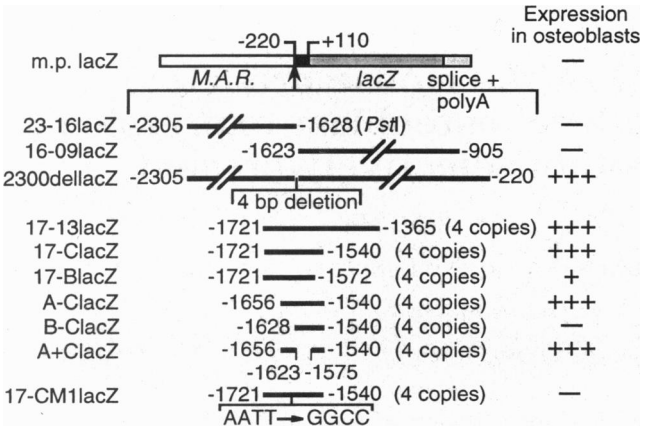

FIG. 1. Schematic representation of DNA constructs containing the *lacZ* reporter gene used to generate transgenic mice. Promoter segments present in m.p.lacZ, 23-16lacZ, and 16-09lacZ were also cloned upstream of the luciferase reporter gene (m.p.lucif, 23-16 lucif, 16-09 lucif, respectively). *M.A.R.*, matrix attachment locus of the chicken lysozyme locus.

β-D-galactoside (X-Gal). All embryos positive by X-Gal staining were studied by histology.

Reporter Gene Expression. Expression of the β-galactosidase gene in day-15.5-p.c. embryos was assessed as described (2). Luciferase activity was measured in 1-month-old mice as described in detail elsewhere (2).

Cell Culture. ROS17/2 rat osteosarcoma cells, RAG mouse renal adenocarcinoma cells, C3H/10T½ mouse fibroblasts, EL4 mouse lymphoma cells, S194/5.XXO-1 mouse myeloma cells, RCS rat chondrosarcoma cells, and C2C12 mouse myoblasts were cultured in Dulbecco's modified minimal essential medium (DMEM) supplemented with 10% fetal calf serum and antibiotics. NIH 3T3 mouse fibroblasts and BALB/3T3 mouse fibroblasts were cultured in DMEM supplemented with 10% calf serum and antibiotics. MC3T3-E1 mouse osteoblasts were cultured in α minimal essential medium supplemented with 10% fetal calf serum and antibiotics.

Gel Retardation Assays. Nuclear extracts were prepared from 80% confluent cells as described (9), except that the buffer used to extract nuclear proteins contained 0.55 M NaCl.

Ten femtomoles of ds gel-purified oligonucleotide probes labeled with the Klenow fragment of *E. coli* DNA polymerase I and [α-^{32}P]dCTP were incubated for 30 min at room temperature in the presence of 5–8 μg of nuclear proteins, 4 μg of poly(dI-dC)·poly(dI-dC), 20 mM Hepes (pH 7.9), 50 mM or 100 mM KCl, 1 mM EDTA, 0.5% Nonidet P-40, and 10% glycerol. The reaction mixture was fractionated by electrophoresis on a 5% polyacrylamide gel. Competition experiments were performed in the presence of a 10- to 100-fold excess of competitor.

RESULTS

Transgenic Mice for m.p.lacZ. and m.p.lucif. We cloned the −220 to +110 segment of the mouse pro-α1(I) proximal promoter upstream of the *lacZ* gene and of the luciferase gene in separate constructions (m.p.lacZ, and m.p.lucif, respectively) and generated four transgenic mice that harbored both transgenes and expressed the luciferase gene. In 1-month-old mice, all four transgenic lines expressed the luciferase gene at very low levels, mainly in skin but not in bones (Table 1). The extremely low levels of expression observed in vertebrae with lines 17.4 and 17.24 could have been due to expression of the luciferase gene in tendons contaminating the vertebrae preparation. None of the transgenic embryos stained positive by X-Gal at day 15.5 p.c., a time at which osteoblasts were strongly stained with X-Gal in embryos harboring a 2.3-kb segment of the pro-α1(I) proximal promoter (2). These results suggested that the −220-bp to +110-bp segment of the pro-α1(I) gene had little or no activity by itself in transgenic mice and, hence, that this promoter could be used to functionally delineate potential upstream osteoblast-specific cis-acting elements in the pro-α1(I) promoter.

Transgenic Mice for 23-16lacZ, 23-16lucif, 16-09lacZ, and 16-09lucif. Previous results obtained with transgenic mice strongly suggested that the −2.3-kb to −0.9-kb segment of the mouse pro-α1(I) proximal promoter contained a cis-acting element responsible for high-level osteoblast- and odontoblast-specific expression of reporter genes (2). This fragment was divided into two segments, one extending from −2305 to −1628 and the other from −1623 to −905. Each of these two fragments was cloned upstream of the minimal 220-bp promoter in separate chimeric constructions with the luciferase and the *lacZ* genes (23-16lucif and 23-16lacZ for the −2305 to −1628 fragment and 16-09lucif and 16-09lacZ for the −1623 to −905 fragment, respectively). Transgenic mice were generated harboring two identical promoter constructions with the *lacZ* and luciferase reporter genes (23-16lacZ + 23-16lucif; 16-09lacZ + 16-09lucif). Seven 1-month-old mice harboring the 23-16lucif and 23-16lacZ constructs and five 1-month-old mice harboring the 16-09lucif and 16-09lacZ constructs showed luciferase expression in skin, but except for one line (line 3.52), little expression of the transgene was found in other tissues including bones (Table 1). The higher levels of luciferase activity observed in vertebrae for line 3.52 could have been due to higher levels of expression of the luciferase gene in adhering ligaments and in skin. None of these 12 transgenic lines showed X-Gal staining in osteoblasts during embryonic development. These results raised the possibility that the osteoblast-specific element might include sequences located on both sides of the −1625 *Pst* I cleavage site used to generate the 23-16 and 16-09 chimeric constructions.

Transgenic Mice for 2300dellacZ. Transgenic mice harboring 2.3 kb of the pro-α1(I) proximal promoter with a 4-bp deletion in the −1625 *Pst* I site (2300dellacZ) were generated. Twelve day-15.5-p.c. embryos harbored the reporter gene. Seven showed identical patterns of strong X-Gal staining of ossification centers in whole-mount embryos (Fig. 2*A*) and of osteoblasts in histological sections (data not shown). The other five did not express the *lacZ* transgene. We concluded that the

Table 1. Luciferase activity in tissue extracts of 1-month-old transgenic mice harboring m.p.lucif, 23-16lucif, or 16-09lucif

	m.p.lucif				23-16lucif							16-09lucif				
Tissue	17.4	17.21	17.24	17.26	4.1	4.18	4.23	4.25	4.33	4.51	4.57	3.6	3.18	3.27	3.47	3.52
Vertebrae	1	UD	0.3	UD	0.3	4	0.02	0.2	0.6	0.4	7	0.6	1.5	UD	1	22
Calvarium	UD	UD	UD	UD	UD	UD	0.02	0.3	0.2	0.2	1	UD	UD	UD	UD	3
Femur	UD	UD	UD	UD	UD	UD	0.1	0.2	0.2	0.2	0.3	UD	UD	UD	UD	1
Skin	10	4	4	3	66	17	15	42	57	19	92	2	37	10	9	1195
Tendon	3	UD	4	UD	UD	UD	0.3	0.7	2	1	16	3	8	UD	11	103
Liver	UD	UD	UD	UD	UD	0.08	0.03	0.06	0.04	0.04	0.09	UD	UD	UD	UD	0.1
Brain	1	UD	UD	0.05	4.9	0.06	0.05	0.01	0.2	0.2	0.6	UD	16	UD	0.01	1.4

Luciferase activities are expressed as 10^3 light units per mg of protein. UD, undetectable.

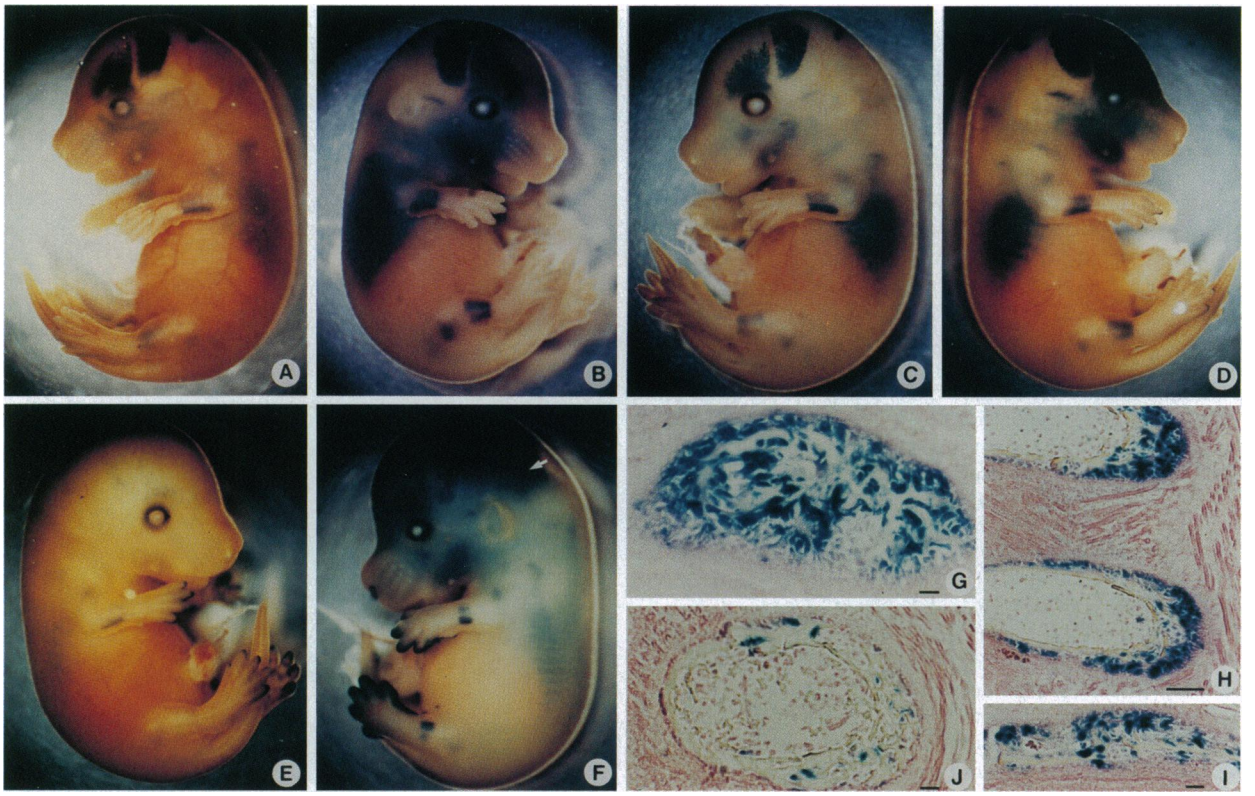

FIG. 2. Expression of the *lacZ* gene in day-15.5-p.c. embryos, as indicated by X-Gal staining. (*A–F*) X-Gal staining of whole-mount embryos harboring 2300dellacZ (*A*), 17-13lacZ (*B*), 17-ClacZ (*C*), A-ClacZ (*D*), 17-BlacZ (*E*), and A+ClacZ (*F*). For each promoter construction, identical patterns were observed in several founder embryos. Each of the panels shows staining of the ossification centers. In *E* the staining is fainter than in embryos harboring the other constructions, but the overall pattern is the same. In *D* and *E*, in addition to staining of the ossification centers, the tips of the digits are stained. In *F*, in addition to staining of the ossification centers, staining of the sides of the digits and of areas in the brain (arrow) was detected. (*G*) Transverse section of the clavicle of an embryo harboring the 17-13lacZ construct, showing expression of the *lacZ* gene in osteoblasts. (*H*) Section of the ribs of an embryo harboring the A-ClacZ construct showing X-Gal staining of osteoblasts but not of muscle fibroblasts or of chondrocytes. (*I*) Sagittal section of the frontal bone of an embryo harboring the A-ClacZ construct showing staining of osteoblasts. (*J*) Transverse section of a rib of an embryo harboring the 17-BlacZ construct showing staining of osteoblasts; this staining is fainter than the staining observed with the other constructs. (Bars = 200 μm.)

−1627 to −1624 sequence was not needed for osteoblast-specific expression of the *lacZ* gene.

Transgenic Mice for 17-13lacZ. We next used a construct containing the −1721 to −1366 segment (17-13lacZ) to generate transgenic mice. Out of eight day-15.5-p.c. transgenic embryos, two were not stained. Six expressed the *lacZ* gene specifically and at high levels in ossification centers (Fig. 2*B*); two of these also showed a staining of the tips of the digits (data not shown). Histological sections confirmed that the staining was restricted to osteoblasts (Fig. 2*G*) and in two cases to some discrete cells of finger tips (data not shown). This result demonstrated that a 356-bp segment cloned upstream of the proximal 220-bp pro-α1(I) promoter was able to induce high levels of expression of the *lacZ* reporter gene in osteoblasts.

Transgenic Mice for 17-ClacZ, 17-BlacZ, A-ClacZ, and B-ClacZ. To define the 3′ boundary of the segment required for osteoblast-specific transgene expression, we first used a −1721 to −1540 segment (17-ClacZ) to generate transgenic mice. Seven day-15.5-p.c. transgenic embryos were obtained. Three showed strong staining of ossification centers in whole-mount embryos (Fig. 2*C*) completely restricted to osteoblasts in histological sections (data not shown), whereas the other four were not stained by X-Gal. We then used a construct containing a −1721 to −1572 segment to generate transgenic mice (17-BlacZ). Fifteen transgenic day-15.5-p.c. embryos were obtained. Three of these day-15.5-p.c. embryos showed faint X-Gal staining specifically in ossification centers and finger tips (Fig. 2*E*). Histological examination confirmed that

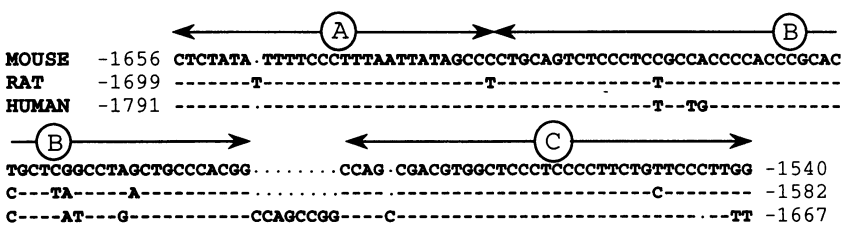

FIG. 3. Minimal sequence of the mouse pro-α1(I) collagen promoter that induced high-level expression of the *lacZ* gene in osteoblasts of transgenic mice, when cloned upstream of the 220 bp of the proximal pro-α1(I) promoter. This sequence can be divided into three segments (A, B, and C), which have different functions (see text for details). The corresponding sequences of the human pro-α1(I) promoter (GenBank accession no. U06669) and of the rat pro-α1(I) promoter (GenBank accession no. J04464) are also indicated (–, identical nucleotide; ·, missing nucleotide). Note that the mouse sequence differs from the sequence published previously (GenBank accession no. X54876) at bases −1564, −1575, and −1576.

the *lacZ* gene was expressed in osteoblasts and showed some heterogeneity in the intensity of staining, which was not observed with other constructs (Fig. 2*J*). Three other embryos expressed the *lacZ* gene, one in finger tips, one in some cartilages, and one in the nasal area, but none of them displayed osteoblast staining. Nine embryos were not stained by X-Gal.

The same strategy was used to delineate the 5′ boundary of the osteoblast-specific element. We first used a 117-bp segment extending from −1656 to −1540 to generate transgenic mice (A-ClacZ). Seven day-15.5-p.c. transgenic embryos were obtained. Four expressed the reporter gene at high levels in ossification centers in whole-mount embryos (Fig. 2*D*) and in osteoblasts in histological sections (Fig. 2 *H* and *I*). In addition, the tips of the fingers were stained by X-Gal in all four embryos (Fig. 2*D*). The other three embryos were not stained by X-Gal. A shorter segment of the promoter, extending from −1628 to −1540 (B-ClacZ), was then used to generate transgenic mice. None of eight day-15.5-p.c. transgenic embryos expressed the reporter gene.

We concluded that the 117-bp segment extending from −1656 to −1540 was the minimal segment necessary and sufficient to confer consistently high levels of expression of the *lacZ* gene in osteoblasts, when cloned upstream of the proximal 220-bp pro-α1(I) promoter. This 117-bp segment can be divided into three subsegments (Fig. 3). The A segment, located between −1656 bp and −1628 bp, was necessary to obtain osteoblast-specific expression of the reporter gene, whereas the C segment extending from −1575 bp to −1540 bp was not strictly required for osteoblast-specific expression of the *lacZ* gene but was necessary to obtain high levels of osteoblast-specific expression.

Transgenic Mice for A+ClacZ. To define the role of the B subsegment of the 117-bp element (Fig. 3), transgenic mice harboring only the A and the C sequences (A+ClacZ) were generated. Out of seven day-15.5-p.c. transgenic embryos, six expressed the *lacZ* gene in ossification centers (Fig. 2*F*), and this expression corresponded to osteoblast staining in histological sections (data not shown). In addition, all six embryos expressed the *lacZ* gene along the fingers and in discrete areas of the brain and spinal cord (Fig. 2*F*). These results suggested that the B segment was dispensable for osteoblast-specific promoter activity. In transgenic mice, the role of the B segment could be to inhibit expression of the *lacZ* gene in some discrete areas. Another possibility that could account for the promiscuous expression of the A+ClacZ construct is that the junction of the A and C elements through a *Pst* I site created a new binding site for a neuronal DNA-binding protein.

Gel Shift Experiments. Gel shift assays were performed using nuclear extracts from various cell types and ds oligonucleotides from −1656 to −1614 (oligo 1; A segment plus 14 bp of B segment), from −1649 to −1625 (oligo 2; part of A segment), and from −1576 to −1540 (oligo 3, C segment). With oligo 1 the pattern of DNA–protein complexes was identical with nuclear extracts from two osteoblastic cell lines (ROS17/2 cells and MC3T3-E1 cells) but different from those obtained with nuclear extracts from all nonosteoblastic cell lines (Fig. 4*A*). Specifically, complex 3 in Fig. 4*A* was only seen with nuclear extracts from osteoblastic cells. Another complex (complex 6) was seen with nuclear extracts of both osteoblasts and fibroblasts. The same DNA–protein complexes were also observed when oligo 2 was used as a probe (data not shown). When oligo 3 was used, an identical pattern of DNA–protein complexes was observed with nuclear extracts from the various cell types (data not shown). Hence, our DNA-binding assays suggest that an osteoblast-specific protein and a protein specific for type I collagen-producing cells bind to the sequence that, in transgenic mice, is required to confer osteoblast-specific expression to a *lacZ* reporter gene.

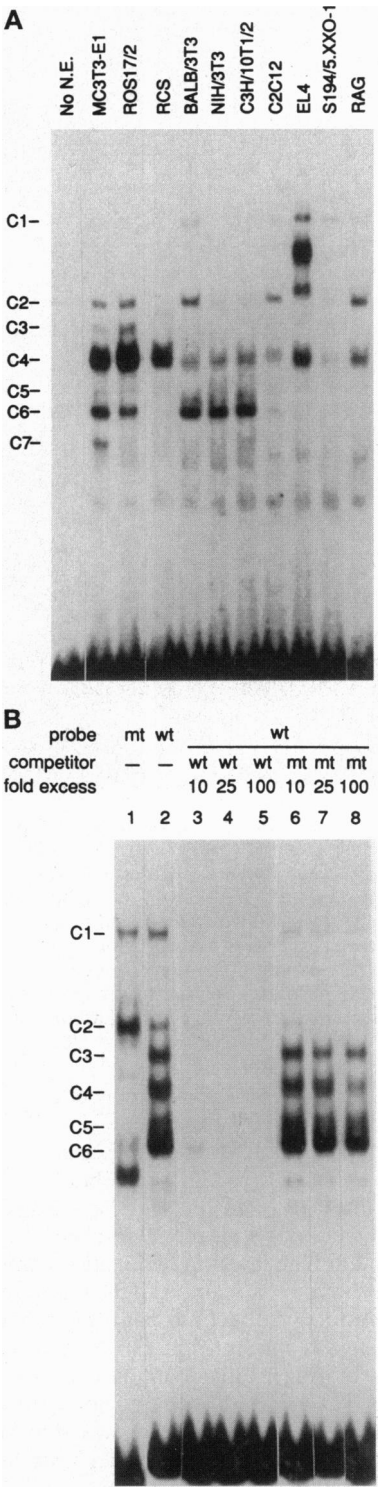

FIG. 4. Identification of an osteoblast-specific DNA–protein complex. (*A*) Gel shift experiment with a −1656 to −1614 ds oligonucleotide (oligo 1) and nuclear extracts from different cell lines. (*B*) A 4-bp mutation (AATT to GGCC) in oligo 1 abolished complexes 3–6 with nuclear extract of ROS17/2 cells. Lane 1, labeled mutant ds oligonucleotide (mt); lane 2, labeled wild-type ds oligonucleotide (wt); lanes 3–8, competition experiments using wild-type ds oligonucleotide as probe and the indicated molar excess of wild-type and mutant competitors. N.E., nuclear extract.

Transgenic Mice for 17-CM1lacZ. To confirm the functional role of the A element, transgenic mice harboring the 17-CM1lacZ construct were generated. This construct was identical to the 17-ClacZ construct that conferred high levels of

lacZ gene expression specifically in osteoblasts, except that four bases located in the A element (between −1636 and −1639) were mutated from AATT to GGCC. In gel shift assays, this 4-bp mutation abolished the binding of several DNA–protein complexes, including the osteoblast-specific complex 3 (Fig. 4*B*). None of the six transgenic mice harboring this construction showed any X-Gal staining at day 15.5 p.c. This confirmed the central role of the A segment for osteoblast-specific expression.

DISCUSSION

Our deletion analysis of the mouse pro-α1(I) collagen promoter in transgenic mice demonstrates that a 117-bp segment, located between −1656 bp and −1540 bp, confers high-level expression of a *lacZ* reporter gene selectively in osteoblasts when cloned upstream of the proximal 220-bp mouse pro-α1(I) promoter. Previous data showing that a 2295-bp rat pro-α1(I) proximal promoter was active in bones and tendons whereas a 1672-bp proximal promoter was inactive are consistent with this result (6). Delineation of this short osteoblast-specific element further supports our previous hypothesis of a modular arrangement of separate cis-acting sequences in the pro-α1(I) collagen gene, which direct expression of the gene in different type I collagen-producing cells (2). Mice harboring this 117-bp sequence also showed X-Gal staining at the distal tip of the digits, but no other nonosteoblastic expression was detected. It is possible that the transcription factor(s) that would be mainly responsible for activating the gene in osteoblasts, or a close relative of it, is expressed in finger tips at 15.5 days p.c.. Since expression in finger tips was not observed with a 2.3-kb promoter, it is likely that other elements in the promoter are able to suppress this expression.

The 117-bp segment responsible for high-level osteoblast-specific expression of the *lacZ* gene in transgenic mice contained two sequences that appeared to have different functions (Fig. 3). The A sequence, located between −1656 and −1628, was required to obtain expression of the *lacZ* gene in osteoblasts, whereas the C sequence, located between −1575 and −1540, was essential to obtain consistent and high-level expression of the *lacZ* gene in osteoblasts. DNA-binding assays showed that the A sequence bound an osteoblast-specific protein (C3 in Fig. 4), while the G+C-rich C segment bound proteins present in extracts of different cell types including SP1 (data not shown). Introduction of a 4-bp mutation in the A element that abolished binding of the osteoblast-specific protein also abolished osteoblast-specific expression in transgenic mice. This suggests a model where the binding of an osteoblast-specific protein to the A element and of ubiquitous DNA-binding proteins to the C element activates the pro-α1(I) collagen gene in osteoblasts (Fig. 5). This model is based on two types of complementary results, which were obtained in intact animals and by *in vitro* DNA binding studies. The 4-bp mutation also abolished formation of at least two other complexes in gel shift assays (C4 and C6 in Fig. 4), which were not osteoblast specific. It is likely that other proteins besides the osteoblast-specific protein also bind to the part of the A segment defined by the 4-bp mutation.

The A element contains an ATTAT motif, which suggests that it could bind a homeoprotein (10). A putative binding site for a homeoprotein, designated an OC box, has also been implicated in the activation of the osteocalcin gene in osteoblasts (11). However, activation of the osteocalcin gene in osteoblasts occurs later in osteoblast differentiation than that of the pro-α1(I) collagen gene. Two other DNA segments in the osteocalcin promoter have also been shown to be involved in osteoblast-specific expression in transient expression experiments, but their sequences show no homology with the sequence of the A element (12). We speculate that the osteoblast-specific DNA-binding protein binding to the A element might help in better defining the molecular mechanisms responsible for the differentiation of mesenchymal cells into osteoblasts.

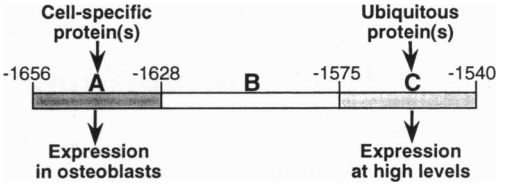

FIG. 5. Model representing the separate postulated roles of the A and C segments in conferring high-level osteoblast-specific expression of the pro-α1(I) collagen gene. See *Discussion* for details.

We thank Drs. Farach-Carson, J.H. Kimura, and R. McKnight for the generous gift of ROS17/2 cells and MC3T3-E1 cells, of RCS cells, and of the chicken lysozyme gene matrix-attachment region, respectively. We thank Drs. S. Maity and V. Lefebvre for critique of the manuscript. DNA sequencing was performed by the DNA Core Sequencing Facility at M. D. Anderson Cancer Center, which was supported by National Cancer Institute Grant CA16672. This work was supported by Grants HL41264 and AR42909 from the National Institutes of Health (to B.d.C.). J.A.R. was the recipient of fellowships from the Mérieux Foundation, the European Dialysis and Transplant Association, and the University Cancer Foundation, M.D. Anderson Cancer Center.

1. Siddhanti, S. R. & Quarles, L. D. (1994) *J. Cell. Biochem.* **55,** 310–320.
2. Rossert, J., Eberspaecher, H. & de Crombrugghe, B. (1995) *J. Cell Biol.* **129,** 1421–1432.
3. Liska, D. A. J., Reed, M. J., Sage, E. H. & Bornstein, P. (1994) *J. Cell Biol.* **125,** 695–704.
4. Pavlin, D., Lichtler, A. C., Bedalov, A., Kream, B. E., Harrison, J. R., Thomas, H. F., Gronowicz, G. A., Clark, S. H., Woody, C. O. & Rowe, D. W. (1992) *J. Cell Biol.* **116,** 227–236.
5. Bedalov, A., Breault, D. T., Sokolov, B. P., Lichtler, A. C., Bedalov, I., Clark, S. H., Mack, K., Khillan, J. S., Woody, C. O., Kream, B. E. & Rowe, D. W. (1994) *J. Biol. Chem.* **269,** 4903–4909.
6. Bogdanovic, Z., Bedalov, A., Krebsbach, P. K., Pavlin, D., Woody, C. O., Clark, S. H., Thomas, H. F., Rowe, D. W., Kream, B. E. & Lichtler, A. C. (1994) *J. Bone Miner. Res.* **9,** 285–292.
7. Phi-Van, L. & Strätling, W. H. (1988) *EMBO J.* **7,** 655–664.
8. McKnight, R. A., Shamay, A., Sankaran, L., Wall, R. J. & Hennighausen, L. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6943–6947.
9. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11,** 1475–1489.
10. Ekker, S. C., von Kessler, D. P. & Beachy, P. A. (1992) *EMBO J.* **11,** 4059–4072.
11. Hoffmann, H. M., Catron, K. M., van Wijnen, A. J., McCabe, L. R., Lian, J. B., Stein, G. S. & Stein, J. L. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12887–12891.
12. Ducy, P. & Karsenty, G. (1995) *Mol. Cell. Biol.* **15,** 1858–1869.