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PTH-Responsive Osteoblast Nuclear Matrix Architectural Transcription Factor Binds to the Rat Type I Collagen Promoter

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Abstract In connective tissue, cell structure contributes to type I collagen expression. Differences in osteoblast microarchitecture may account for the two distinct cis elements regulating basal expression, in vivo and in vitro, of the rat type I collagen α1(I) polypeptide chain (COL1A1). The COL1A1 promoter conformation may be the penultimate culmination of osteoblast structure. Architectural transcription factors bind to the minor groove of AT-rich DNA and bend it, altering interactions between other trans-acting proteins. Similarly, nuclear matrix (NM) proteins bind to the minor groove of AT-rich matrix-attachment regions, regulating transcription by altering DNA structure. We propose that osteoblast NM architectural transcription factors link cell structure to promoter geometry and COL1A1 transcription. Our objective was to identify potential osteoblast NM architectural transcription factors near the in vitro and in vivo regulatory regions of the rat COL1A1 promoter. Nuclear protein-promoter interactions were analyzed by gel shift analysis and related techniques. NM extracts were derived from rat osteosarcoma cells and from rat bone. The NM protein, *NMP4,* and a soluble nuclear protein, *NP,* both bound to two homologous poly(dT) elements within the COL1A1 in vitro regulatory region and proximal to the in vivo regulatory element. These proteins bound within the minor groove and bent the DNA. Parathyroid hormone increased *NP/NMP4* binding to both poly(dT) elements and decreased COL1A1 mRNA in the osteosarcoma cells. *NP/NMP4*-COL1A1 promoter interactions may represent a molecular pathway by which osteoblast structure is coupled to COL1A1 expression. J. Cell. Biochem. 69:336–352. © 1998 Wiley-Liss, Inc.

Key words: architectural transcription factor; nuclear matrix; osteoblast; parathyroid hormone; type I collagen

The expression of type I collagen in connective tissue is regulated in part by cell structure [Dhawan et al., 1991; Benya and Shaffer, 1982; Diegelmann and Peterkofsky, 1972; Scherft and Heersche, 1975]. The transcription rate and mRNA half-life of the rat type I collagen a1(I) polypeptide chain (COL1A1) were decreased in suspended as compared to adherent Swiss 3T3 fibroblasts, which was interpreted as a coupling of these processes to cell architecture [Dhawan et al., 1991]. Similarly, the morphology of rabbit articular chondrocytes correlated with the expression of type I and type II collagen; flat adherent cells produced type I collagen, and round, suspended cells expressed type II collagen [Benya, 1988; Benya and Shaffer, 1982). In osteoblasts, distinct cis-regulatory regions of the rat type I collagen α1(l) polypeptide chain (COL1A1) promoter, over 600 nucleotides (nt) apart, mediated basal transcription in bone and in culture; this was attributed to differences in cell microarchitecture [Dodig et al., 1996; Bedalov et al., 1995; Bogdanovic et al., 1994]. Basal expression of COL1A1 promoter fusion genes in vivo was mediated by an element between -1683/-1670 nt [Dodig et al., 1996]. However, the region between -3518/-2295 nt governed expression in osteoblast cul-

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tures derived from these transgenic mice and in osteoblastic cell lines [Dodig et al., 1996; Bedalov et al., 1995; Bogdanovic et al., 1994]. Finally, osteoblast response to parathyroid hormone (PTH) may be mediated in part by cell structure, as evidenced by the hormone's dramatic effect on cyto- and nucleoskeletal organization [Torrungruang et al., 1998; Bidwell et al., 1996; Bronner and Stein, 1992; Egan et al., 1991; Ali et al., 1990; Lormi and Marie, 1990; Ferrier et al., 1988; Aubin et al., 1983].

The osteoblast tissue matrix provides a molecular framework for the regulation of gene expression through changes in cell architecture. The tissue matrix consists of the dynamic linkages between the skeletal networks of the nucleus (the nuclear matrix), the cytoplasm (the cytoskeleton), and the extracellular matrix that link the DNA with the cell periphery [Pienta and Hoover, 1994]. The nuclear matrix is a unique biochemical fraction that consists of the lamin-nuclear pore complex, an internal fibrillogranular network of ribonuclear proteins, and the chromosome scaffold [Davie, 1995; Nickerson et al., 1995].

Although the molecular mechanisms that couple cell structure to transcription are not known, promoter conformation may be the penultimate culmination of cyto-and nucleoskeletal organization. Architectural transcription factors regulate gene expression by bending DNA, thereby modulating the interactions between other trans-acting proteins [Wolffe, 1994]. These proteins often recognize an anomalous structural feature of the double helix such as the narrow minor groove of AT-rich sequences [Churchill and Travers, 1991]. Similarly, nuclear matrix proteins bind to the minor groove of AT-rich matrix-attachment regions (MARs), those DNA elements that fasten the chromosomes to the nuclear scaffold [Davie, 1995; Boulikas, 1995; Pienta et al., 1991; Phi-Van et al., 1990; Mirkovitch et al., 1984]. Some of these MAR-binding nuclear matrix proteins regulate transcription, putatively by the relief of superhelical torsional strain [Nakagomi et al., 1994; Forrester et al., 1994; Bode et al., 1992].

We propose that osteoblast nuclear matrix architectural transcription factors link cell structure to promoter geometry and COL1A1 transcription. Osteoblast nuclear matrix–DNA binding proteins would be exceptionally placed to render alterations in tissue matrix organization into changes in promoter conformation, particularly if their binding sites were within the promoter itself.

Our objective was to identify candidate proteins as osteoblast nuclear matrix architectural transcription factors that associated at or near the in vitro and in vivo regulatory regions of the rat COL1A1 promoter. Previously, we described osteoblast nuclear matrix protein–rat COL1A1 promoter interactions, designated as *NMP3* (-2149/-2106 nt) and *NMP4* (-3518/-3406 nt) [Alvarez et al., 1997]. Here we determined that *NMP4* and a soluble nuclear protein, *NP*, both associated within the minor groove of two homologous poly(dT) elements at or near the COL1A1 regulatory regions and bent the DNA. *NP/NMP4*-COL1A1 interactions were modulated by PTH. These proteins may comprise a nuclear pathway by which osteoblast structure and COL1A1 expression are coupled.

MATERIALS AND METHODS

Reagents

Compounds for the extraction buffers were obtained from the Fisher Chemical Co. (Pittsburgh, PA) and Sigma Chemical Co. (St. Louis, MO). The cleavage reagents methidiumpropyl-EDTA and $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (Sigma) were used for DNA footprinting. Diminazene aceturate (berenil), distamycin A hydrochloride, and mithramycin A (Sigma) were prepared as aqueous stock solutions (10 mM). Rat parathyroid hormone [rPTH(1-34)] was obtained from Bachem Bioscience, Inc. (King of Prussia, PA), and diluted in 10 mM acetic acid and acidified saline, respectively. Oligonucleotides were purchased from Gibco-BRL (Grand Island, NY). Isotopes (γ-^{32}PATP and α-^{32}PdNTP) were obtained from NEN (Boston, MA).

Cell Culture

The rat osteosarcoma cells, UMR 106-01, a generous gift from Dr. Nicola Partridge (St Louis University, St Louis, MO), were grown in MEM medium supplemented with 100 IU/ml penicillin, 100 µg/ml streptomycin, 25 µg/ml amphotericin, 2 mM L-glutamine (Gibco-BRL), and 10% fetal bovine serum (FBS) (Sigma). ROS 17/2.8 rat osteosarcoma cells, kindly donated by Dr. Gideon and Dr. Sevgi Rodan (Merck Research Laboratories, West Point, PA), were maintained in Ham's F12 (Gibco-BRL) supplemented with 2.36 g/L $NaHCO_3$, 0.118 g/L $CaCl_2 \cdot 2H_2O$,

TABLE I. Oligonucleotide Probes/Competitors Used for EMSA*

Sequence	Rate COL1A location
5′TTCTTTTTTTTTCTTTTTCT3′	Site A [−3469/−3450 nt]
5′TTATTTTTTTTTCTTTGCCT3′	Site B [−1574/−1555 nt]
5′TCTAGAATATAGAAGCCAAGGATTTCAA3′	Competitor (−3518/−3488 nt)[a]
5′GGGTTTCCTTTTCTCTCTTCTTCTTTTTTTTTCTTTTTCTTTT-TCCTGAGATGGAG3′	Site A [−3489/−3434 nt]
5′TTTCCTTTTGTAGCCCTGACTGTCCTGG3′	Competitor (−3435/3406 nt)[a]
5′TTATTTTTTTTTCTTTGCCT3′	WT (wild-type) Site B (nt) [−1574/−1555
5′TTATTTT**G**T**A**TTCTTTGCCT3′	MUT1
5′TTATTTT**G**T**G**TTCTTTGCCT3′	MUT2
5′TTATTTT**GGG**TTCTTTGCCT3′	MUT3
5′TTA**G**TTT**G**TTT**G**CTTTGCCT3′	MUT4
5′TTCTTTTGTATTCCTTTTCT3′	BCE-1

*The bold letters in the sequences represent mutations to the wild sequence. Similar mutations were made in the 20 bp fragment spanning site A (−3469/−3450 nt, data not shown).
[a]Used as competitor only. All other oligonucleotides used as probes and competitors.

6.106 g/L HEPES, and penicillin, streptomycin, amphotericin, glutamine, and FBS as described above. All cells were maintained in humidified 95% air/5% CO_2 at 37°C.

PTH Treatment

ROS 17/2.8 cells were seeded at a density of ~1.1 × 10^3/cm^2 and grown to near confluence (~ day 7). The cells were treated with either 10 nM rPTH(1-34) or the same volume of vehicle once daily (with a fresh medium change) for 3 days and harvested 24 h after the last treatment.

Protein Isolation

Cell culture. Nuclear matrix proteins were extracted from UMR 106-01 and ROS 17/2.8 cells using a sequential extraction protocol [Alvarez et al., 1997]. Cell plates were placed on ice, the medium removed, and the adherent cell layer washed with ice-cold phosphate buffered saline (PBS). All subsequent steps were performed at 4°C unless specified otherwise. Soluble and membrane proteins were extracted from the cell layer using CSK buffer (100 mM NaCl, 300 mM sucrose, 10 mM PIPES (pH 6.8), 3 mM $MgCl_2$, 1 mM EGTA, 1.2 mM phenylmethylsulfonyl fluoride (PMSF), 0.5% Triton X-100). Upon removal of CSK buffer, the cells were scraped into RSB-Majik buffer (10 mM NaCl, 3 mM $MgCl_2$, 10 mM Tris (pH 7.4), 1.2 mM PMSF, 1% Tween 40, 0.5% sodium deoxycholate) and pelleted by centrifugation. The cell pellet was triturated and rocked in digestion buffer (50 mM NaCl, 300 mM sucrose, 10 mM PIPES (pH 6.8), 3 mM $MgCl_2$, 1 mM EGTA, 0.5% Triton X-100, 1.2 mM PMSF, 100 µg/ml DNaseI, 50 µg/ml RNase A) at room temperature for 20 min. Chromatin proteins were extracted from the digest by the addition of ammonium sulfate to 0.25 M and separated from the nuclear matrix and intermediate filament proteins by centrifugation. The nuclear matrix–intermediate filament pellet was solubilized in disassembly buffer (8 M urea, 20 mM MES (pH 6.6), 1 mM EGTA, 0.1 mM $MgCl_2$, 1.2 mM PMSF, and 1% 2-mercaptoethanol) and dialyzed against assembly buffer (150 mM KCl, 25 mM imidazole-HCl (pH 7.1), 5 mM $MgCl_2$, 0.125 mM EGTA, 2 mM dithiothreitol (DTT), 0.2 mM PMSF) overnight at room temperature. The intermediate filaments reassembled under these conditions and were then separated from the solubilized nuclear matrix proteins upon ultracentrifugation. The nuclear matrix proteins were concentrated, snap-frozen in liquid nitrogen, and stored at −80°C. Nonmatrix, nuclear proteins were obtained from cell lines using a modified standard protocol [Dignam et al., 1983].

Bone. Femurs were stripped of muscle and connective tissue. The epiphyseal cap was then removed, and the adjacent 3 mm section of the metaphyseal bone was resected. The metaphyseal bone segments were minced and trypsinized for 1 h at 37°C. The released cells were pelleted, and nuclear matrix proteins were recovered as described above.

Probe Construction

We used fragments of the rat COL1A1 promoter, -3518/+115 nt (a gift from A. Lichtler, B. Kream, and D. Rowe, The University of Con-

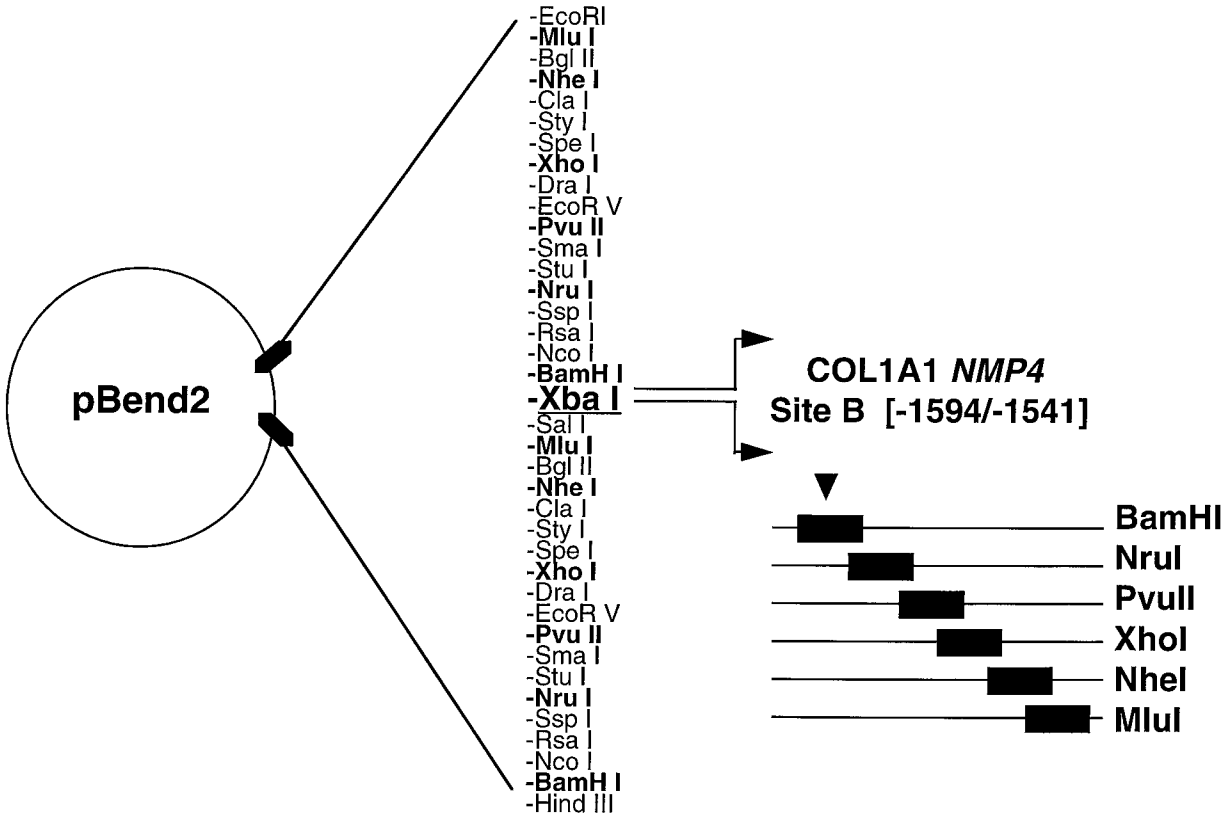

Fig. 1. The pBend2 plasmid used for circular permutation analysis. Details concerning the construction of the plasmid are described in Kim et al. [1989]. *NMP4* binding element, site B, was ligated into the Xba I restriction site to create a pBend2$_B$ plasmid.

necticut Health Center, Farmington, CT) as end-labeled probes in electrophoretic mobility shift assays (EMSA). The 112 base pair (bp) COL1A1 fragment (-3518/-3406 nt) was isolated by gel purification from the XbaI/EcoRI restriction digest. The COL1A1 sequences, site A (-3489/-3434 nt)5′CAAGGGTTTCCTTTTCTCTCTTCTTCTTTTTTTTTCTTTTTCTTTTTCCTGAGATGGAGATGG3' and COL1A1 site B (-1594/-1541 nt)5′TTCTGCTCCCTTGGTCTTTTTTATTTTTTTTTCTTTGCCTTCGTTGCACAAAACTAG3′, were amplified from the promoter using polymerase chain reaction (PCR) and subcloned into pCRTM 2.1 (Invitrogen, San Diego, CA). Sequencing was performed to confirm the fidelity of these subcloned fragments (Exo[-]Pfu Cyclist(tm) DNA Sequencing Kit; Stratagene, La Jolla, CA).

Double-stranded oligonucleotides (dsoligos) were synthesized to various rat COL1A1 promoter regions and to an homologous region within the T-rich, β-casein element (BCE-1) [Schmidhauser et al., 1992], not found on the COL1A1 promoter, for use as probes and competitors in the EMSA experiments (Table I). The 20 bp probes of site A [5′*TTCTTTTTTTTTCTTTTTCT*3′] and site B [5′*TTATTTTTTTTTCTTTGCCT*3′] were generated by end-labeling with [γ-^{32}P] ATP and either used as single-stranded probes or annealed to their complementary oligonucleotides in 100 mM NaCl. Additionally, the 20 bp sequences from -3469/-3450 nt within site A and from -1574/-1555 nt within site B were similarly prepared as wild-type and mutant dsoligos (Table I). Restriction fragment probes were end-labeled with [α-^{32}P]deoxy NTP. All probes were gel purified on 5% TBE polyacrylamide gels, eluted overnight at 37°C, and concentrated using Centricon® Concentrators 10 (Amicon, Beverly, MA).

Electrophoretic Mobility Shift Analysis (EMSA)

Nuclear protein–DNA interactions were characterized using gel shift analysis [Alvarez et al., 1997]. The 20 µl binding reactions included 75 mM KCl, 15% glycerol, 0.15 mM EDTA, 500–2,500 ng of poly(dI)•poly(dC), 0.1 mM DTT, 19 mM HEPES (pH 7.5), 0.0075% NP-40, 2 µg

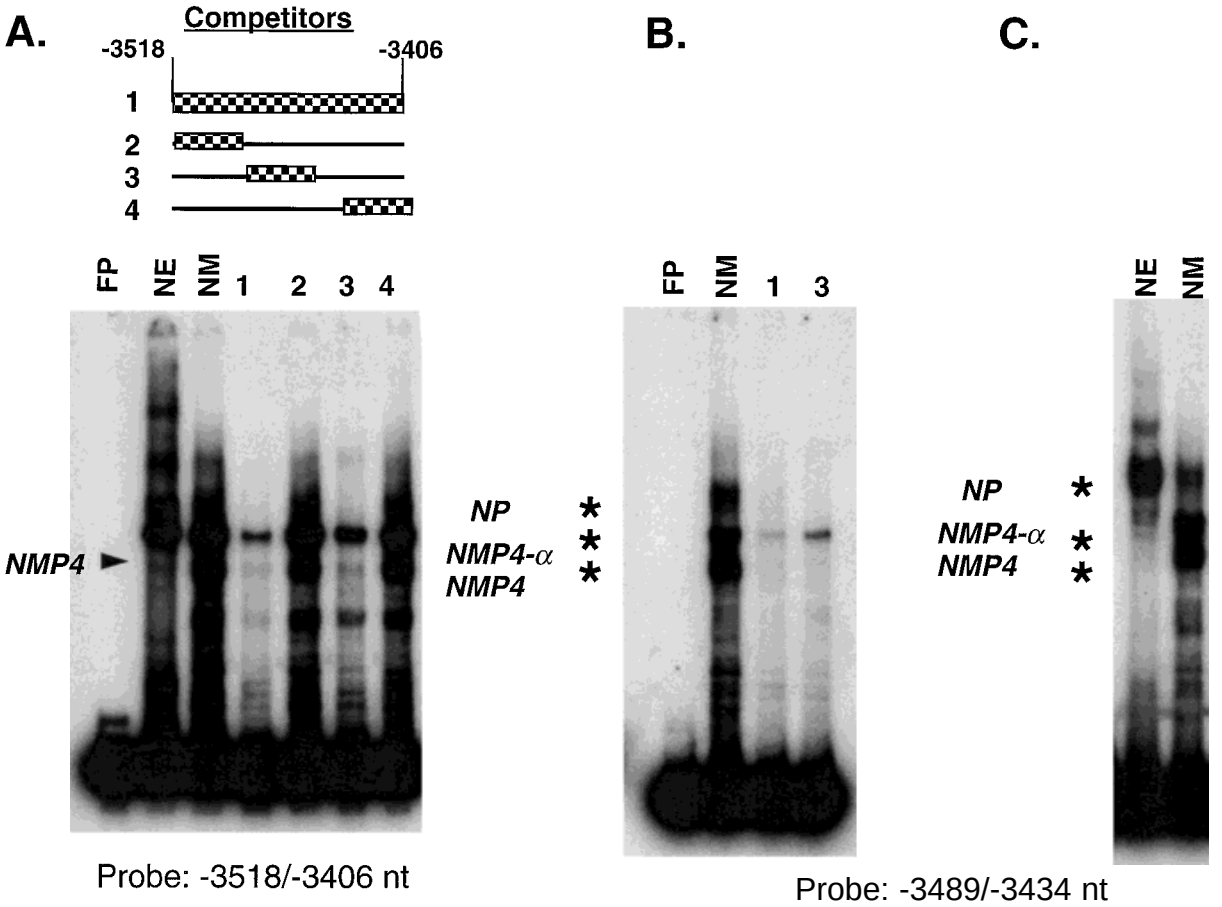

Fig. 2. EMSA to delimit the binding sequence for *NMP4* within the 112 bp region of the rat COL1A1 promoter. Nuclear matrix proteins (NM) and soluble nuclear proteins (NE) from UMR 106-01 cells. FP, free probes. Labeled probes, COL1A1 promoter fragments (112 bp: -3518/-3406 nt (**panel A**); and (55 bp: site A -3489/-3434 nt [**panels B,C**]). EMSA analyses were performed as described in the Materials and Methods. Competitors, unlabeled fragments of the COL1A1 promoter, were added at 200M excess: **lanes 1:** -3518/-3406 nt; **lane 2:** -3518/-3488 nt; **lane 3:** -3489/-3434 nt; **lane 4:** -3435/-3406 nt. **A:** Competition analyses indicated that the *NMP4* binding activity, originally observed between -3518/-3406 nt, was located within -3489/-3434 nt. **B:** The proteins *NMP4, NMP4*-α, and *NP* were recovered using the 55 bp probe (site A, -3489/-3434 nt). **C:** The proteins *NMP4* and NMP4-α were recovered primarily from the nuclear matrix fraction, whereas *NP* was recovered principally from the soluble nuclear fraction. Asterisks denote indicated proteins.

protein, and 0.5 nM end-labeled COL1A1 fragment as probe. These binding reactions were incubated for 30 min at room temperature prior to electrophoresis at 4°C on 5% or 8% polyacrylamide gel (80:1 acrylamide:N,N'methylbisacrylamide) in 1× TGE buffer. The gels were dried under vacuum at 80°C for 1 h and then at room temperature for 30 min. Kodak XAR film was exposed to the gel with an intensifying screen overnight at -80°C.

Densitometry Analysis

Protein-DNA bands detected by ^{32}P-autoradiography were analyzed for intensity and area using the gel documentation system Foto/analyst II and the software Collage(tm) (Fotodyne Inc., Hartland, WI). Images of protein-DNA signals were obtained with a CCD camera and stored as a TIFF file on an Apple Macintosh Centris 650.

Circular Permutation Analysis

Circular permutation analysis was used to determine whether *NMP4* and *NP* bent DNA upon binding to the COL1A1 promoter. Bent DNA can be detected by its anomalous migration in polyacrylamide gels [van der Vliet and Verrijzer, 1993; Kerppola and Curran, 1993; Ferrari et al., 1992; Verrijzer et al., 1991; Wu and Crothers, 1984]. The position of the protein-induced bend in the DNA fragment determines the electrophoretic mobility of the protein-DNA

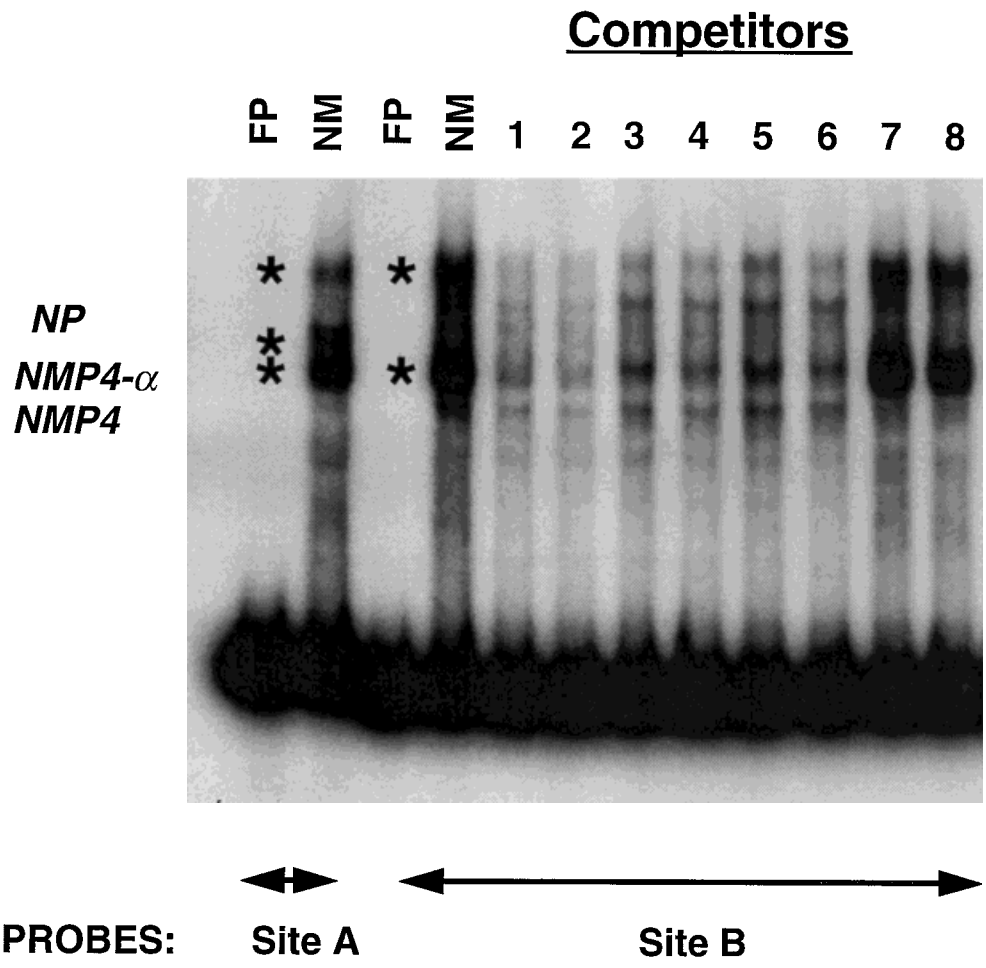

Fig. 3. EMSA identifying a second *NMP4* binding site (site B: -1594/-1541 nt) on the COL1A1 promoter. Nuclear matrix proteins (NM) from UMR 106-01 cells. FP, free probes. Labeled probes, COL1A1 promoter fragments (site A 55 bp: -3489/-3434 nt; site B 53 bp: -1594/-1541 nt). Competitors, unlabeled fragments of the COL1A1 promoter, added at 50/100 M excess, included the following: **lanes 1,2:** -1594/-1541 nt (site B); **lanes 3,4:** -3489/-3434 nt (site A); **lanes 5,6:** -3469/-3450 nt (site A, 20 bp); **lanes 7,8:** the BCE-1, β-casein element 1. The protein NMP4-a was not recovered using the site B probe.

complex, since the mobility of a DNA fragment is dependent on the mean square end-to-end distance [van der Vliet and Verrijzer, 1993; Kerppola and Curran, 1993; Ferrari et al., 1992; Verrijzer et al., 1991]. Reduction of migration is maximal when the bend is located at the center of a DNA fragment and minimal when it is located near the end [van der Vliet and Verrijzer, 1993; Kerppola and Curran, 1993; Ferrari et al., 1992; Verrijzer et al., 1991].

The plasmid pBend2 [Kim et al., 1989] was used to generate several DNA fragments of identical length and nucleotide sequence in which the protein-binding nucleotide sequence (site B, -1594/-1541) was located in circular permutations (Fig. 1). The vector, originally derived from pBR322, contains two identical DNA segments comprised of 17 restriction sites in a direct repeat spanning a central region containing cloning sites (Xba I and Sal I) (Fig. 1) [Kim et al., 1989]. To insert site B into the Xba I cloning site, pBend2 was digested with Xba I and treated with calf intestinal phosphatase. Complementary oligos containing site B were synthesized with an Xba I site on the 5' ends. These oligos were annealed and then ligated into the pBend2 plasmid. Different DNA fragments (all ~180 bp in length) containing site B were generated by digesting pBend2B with the restriction enzymes BamHI, NheI, Xho I, MluI, NruI, and PvuII. The resulting DNA fragments were labeled using Klenow and separated by polyacrylamide gel electrophoresis. These fragments were used in a series of electrophoretic mobility shift assays (EMSA) as described above.

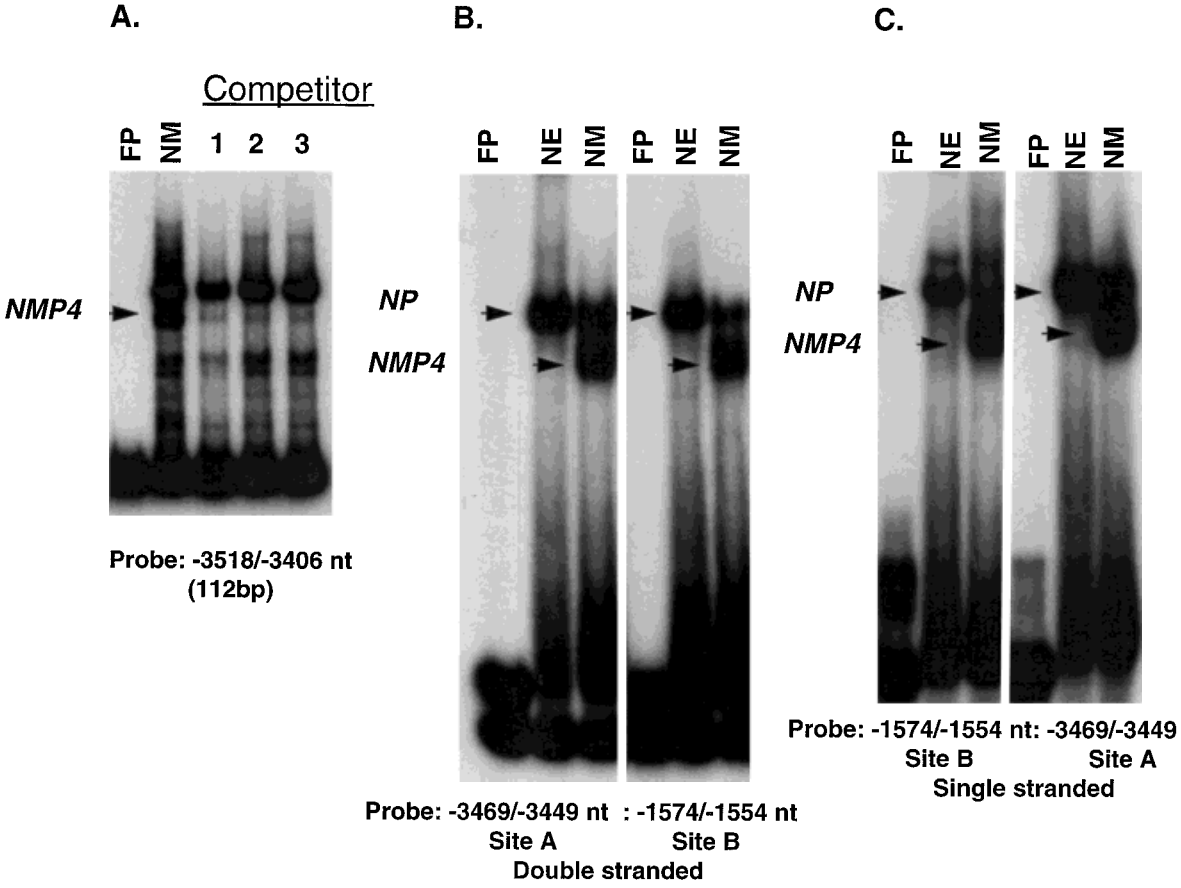

Fig. 4. EMSA analyses to further delimit the *NP/NMP4* binding sites along the rat COL1A1 promoter. FP, free probe; NE, soluble nuclear extracts from UMR-106 cells (2 µg/lane); NM, nuclear matrix extracts from UMR 106-01 cells (2 µg/lane). **A:** Competition analysis indicates that *NP/NMP4* binds between -3469/-3450 nt of the rat COL1A1 promoter. Labeled probe, -3518/-3406 nt. Unlabeled competitors included the following: **lane 1:** rat COL1A1 -3518/-3406 nt (112 bp, 100 M excess); **lane 2:** rat COL1A1 -3469/-3450 nt (20 bp, 100 M excess); **lane 3:** same as lane 2 at 200 M excess. **B:** The double-stranded rat COL1A1 fragments between -3469/-3450 nt (site A) and -1574/-1554 nt (site B) recover *NP/NMP4* binding activity. **C:** The single-stranded T-rich sense strand of the rat COL1A1 promoter between -3469/-3450 nt (site A) and -1574/-1555 nt (site B) recover *NP/NMP4* binding activity.

The degree of *NMP4/NP* bending of the COL1A1 promoter was determined by measuring the DNA flexure angle α. This is defined as the angle by which a segment of the DNA departs from linearity [van der Vliet and Verrijzer, 1993; Kerppola and Curran, 1993; Ferrari et al., 1992; Verrijzer et al., 1991; Kim et al., 1989]. Estimates for α are derived from an empirical relation between the degree of bending and the altered electrophoretic mobility in polyacrylamide gels as described in the following equation [van der Vliet and Verrijzer, 1993; Kerppola and Curran, 1993; Ferrari et al., 1992; Verrijzer et al., 1991; Kim et al., 1989, Thompson and Landy, 1988]:

$$\mu_M/\mu_E = \cos(\alpha/2)$$

In this equation, μ_M is the mobility of the complex with the protein bound at the middle of the fragment, and μ_E is the mobility with the protein bound at the end.

UV Cross-Linking

To prepare BrdU-substituted probes for both sites A and B, we gel-purified and annealed primers to the sense strands of sites A (GGGTTTCCTTTTCTC) and B (TTCTGCTCCCTTGGT) to the antisense oligonucleotides of site A (-3489/-3434) and site B (-1594/-1541), respectively. The sense strand was then filled in using an extension reaction mixture containing dATP, dGTP, BrdU, and α-^{32}PdCTP [Ausubel et al., 1987]. These probes were used in binding

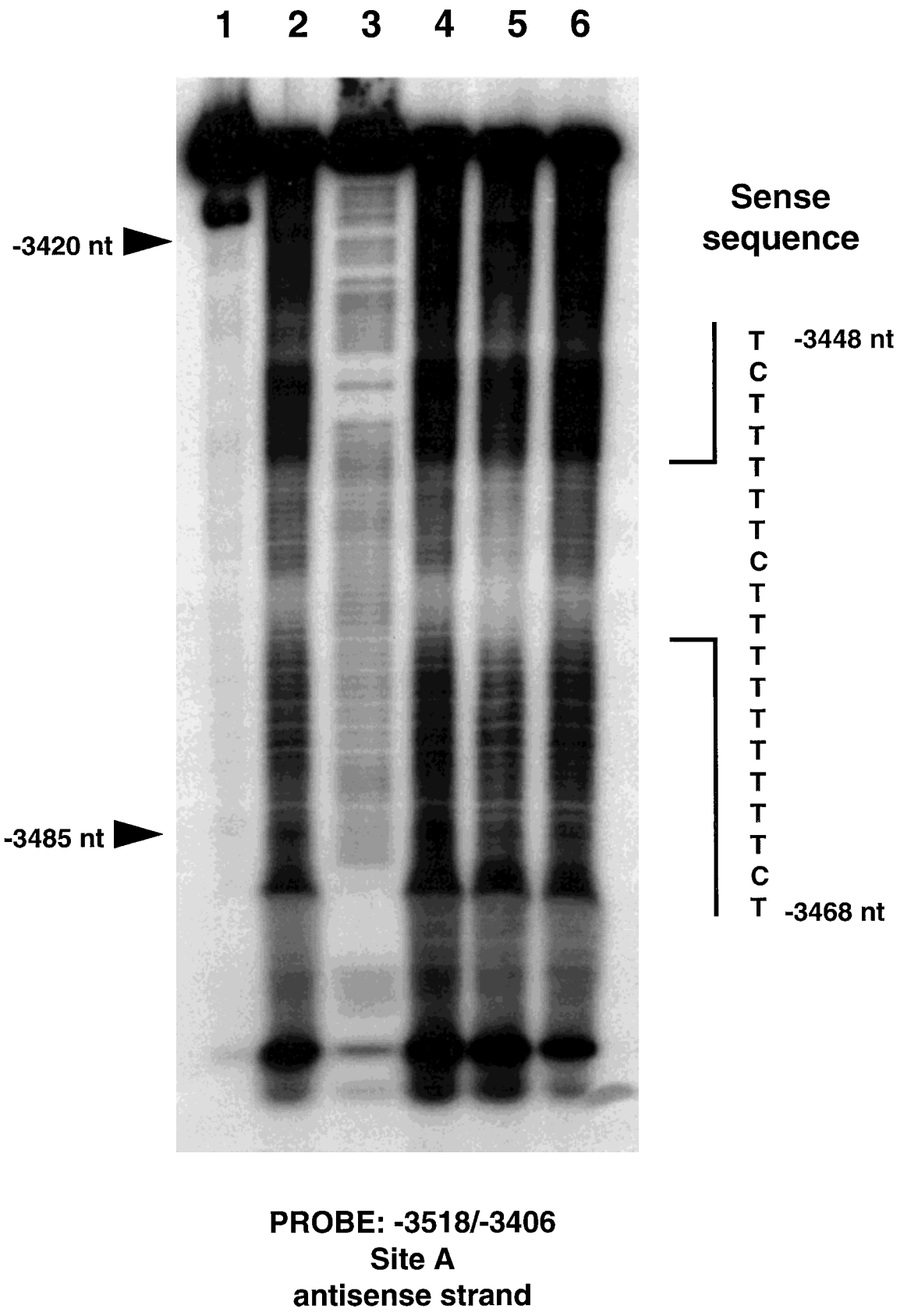

Fig. 5. Methidium propyl EDTA (MPE) footprinting of the antisense strand (-3518/-3406 nt, the 112bp COL1A1 fragment, containing Site A). The nuclear matrix extracts (NM) were obtained from UMR 106-01 rat osteosarcoma cells. **Lane 1:** Free probe (-3518/-3406 nt). **Lane 2:** Probe plus MPE. **Lane 3:** Guanine-adenine ladder**. Lane 4:** Probe/MPE/25 µg NM. **Lane 5:** Probe/MPE/80 µg NM. **Lane 6:** Probe/MPE/80 µg NM plus unlabeled competitor (-3518/-3406 nt, 200 M excess). The footprint for *NMP4/NP* is between -3468/-3448 nt (sense sequence shown). The entire 112 bp fragment is not shown in this figure.

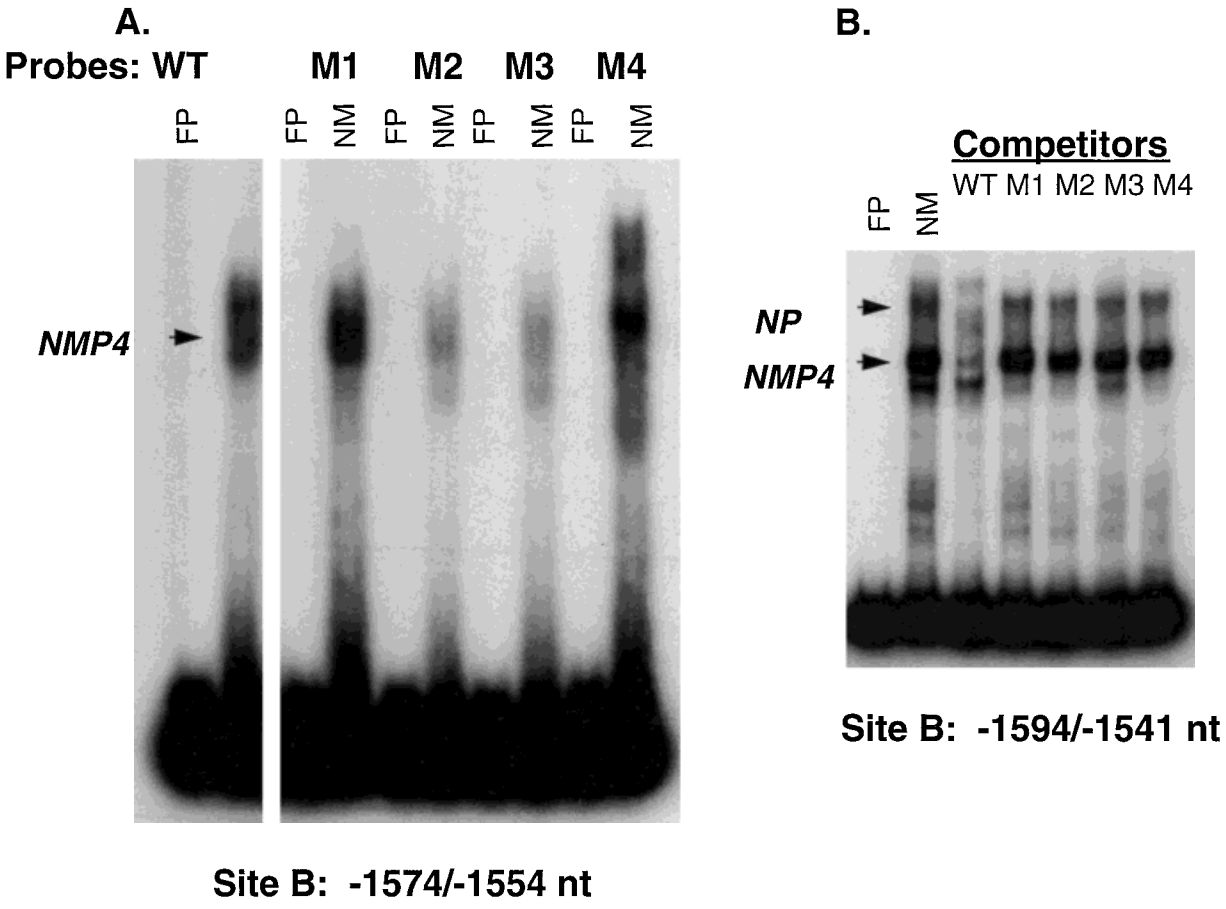

Fig. 6. Mutational analysis of *NMP4* binding site using EMSA. FP, free probe; NM, nuclear matrix extracts from UMR 106-01 cells (2 µg/lane); see Table I for sequences of WT, M1, M2, M3, M4. **A:** Labeled 20 bp probes from site B, -1574/-1554 nt of the rat COL1A1 promoter. The nucleotides -1567/-1565 nt were critical for NP/NMP4 binding. **B:** Labeled 55 bp probe from site B, -1594/-1541 nt of the rat COL1A1 promoter. The unlabeled 20 bp fragments of site B (-1574/-1555 nt, WT-M4) were used as competitors (200 M excess). The mutated fragments did not compete with NMP4 binding.

reactions with nuclear matrix or soluble nuclear extracts. Proteins were cross-linked to the DNA fragments with UV light [Ausubel et al., 1987]. After cross-linking, the binding reactions were digested with DNase I and micrococcal nuclease [Ausubel et al., 1987]. The protein-DNA complexes were electrophoresed on a 10% SDS-polyacrylamide gel, along with a set of molecular weight standards. Controls included binding reactions that were not exposed to UV light, cross-linked extracts incubated with proteinase K, and binding reactions that included specific or nonspecific competitor DNA. The molecular weights of the cross-linked protein-DNA complexes were determined using the gel documentation system Foto/analyst II and the software Collage (Bidwell et al., 1996). Images of the cross-linked protein signal were obtained with a CCD camera and stored as a TIFF file. Molecular weight approximations were based on migration against the calibration standards and fitting the data to various regression models [Bidwell et al., 1996].

MPE Footprinting (Hofmann and Gasser, 1991; Hertzberg and Dervan, 1984)

The restriction fragment -3518 nt to -3406 nt (0.5 nM) was singly end-labeled with Klenow (α-^{32}PdCTP) and incubated with 0.25 mM DTT, 1.25 µg of poly(dI)•poly(dC), and 25–80 µg protein in a total volume of 50 µl of reaction buffer for 25 min at room temperature. Cleavage was initiated by addition of 12.5 µl of (0.1 mM methidium-propyl EDTA (Sigma), 0.1 mM $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (Sigma), 10 mM ascorbic acid (Sigma), 20 mM DTT [Boehringer-Mannheim, Indianapolis, IN]) for 3 min at room temperature. The reaction was terminated upon addition of 250 µl of 2 mM $ZnCl_2$ (Sigma). The reaction products were ethanol-precipi-

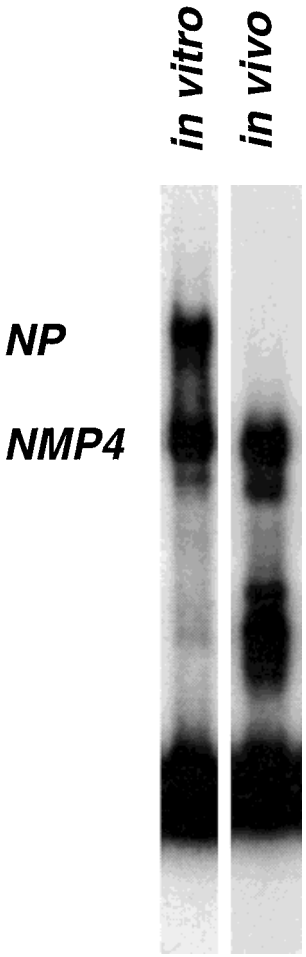

Fig. 7. EMSA to identify in vitro and in vivo *NMP4* binding activity along the rat COL1A1 promoter. Labeled probe, COL1A1 promoter fragment site B (-1594/-1541 nt). Nuclear matrix proteins from UMR 106-01 cells (2 µg/lane, in vitro) and from rat metaphyseal primary spongiosa (4 µg/lane, in vivo). *NP* was not detected in our in vivo preparations. Proteins exhibiting a high mobility were detected in the bone nuclear matrix fractions but not in the extracts from the cell line.

tated, phenol-extracted, and run on a 6% sequencing gel.

Northern Analysis

Total cellular RNA from experimental cell preparations or rat tissue was isolated by lysing the cells with RNAzol B and extraction of the lysate with chloroform. RNA was then processed and analyzed as previously described [Bidwell et al., 1996].

RESULTS

NMP4 Binds to Two Poly(dT) Sites in the Rat COL1A1 Promoter

We have previously reported a nuclear matrix–specific, protein-DNA interaction between -3518/-3406 nt of the rat COL1A1 promoter as *NMP4* [Alvarez et al., 1997]. EMSA of this 112 bp COL1A1 fragment indicated numerous protein-DNA bands including *NMP4* (Fig. 2A). Therefore, to further define the *NMP4* binding sequence within the -3518/-3406 nt region, we performed competition analyses with unlabeled dsoligos spanning the 112 bp labeled probe (Fig. 2A). These data indicated that *NMP4* was binding to the 55 bp fragment (-3489/-3434 nt) containing a poly(dCdT) region:

COL1A1 site A (-3489/-3434 nt):
5′GGGTTTCCTTTTCTCTCTTCTTCTTTTTTT-TTCTTTTTCTTTTTCCTGAGATGGAG3′.

Using this DNA fragment (site A) as a labeled probe, we recovered the prominent *NMP4* binding activity along with another minor, sequence-specific nuclear matrix protein-DNA band, *NMP4*-α, and a protein-DNA interaction we designated as *NP* that was primarily localized in the soluble nuclear fraction (Fig. 2B,C).

A reexamination of the rat COL1A1 promoter sequence revealed another poly(dCdT) site between -1594/-1541 nt, in close proximity to the element that regulates basal expression of COL1A1 in vivo (-1683/-1670 nt [Dodig et al., 1996]). Using this 53 bp fragment (site B) as a probe, we recovered *NMP4* and *NP* binding activities in a gel shift assay as determined by protein mobility and competition with site A (Fig. 3).

COL1A1 site B (-1594/-1541 nt):
5′TTCTGCTCCCTTGGTCTTTTTTATTTTTT-TTTCTTTGCCTTCGTTGCACAAAAC3′.

NMP4-α was not recovered using the labeled probe site B. The regions between -3469/-3450 nt and -1574/-1555 nt are 85% homologous, and both contain a run of nine Ts interrupted by a C followed by three Ts:

Site A: 5′TTCTTTTTTTTTCTTTTTCT3′ (-3469/-3450 nt)
Site B: 5′TTATTTTTTTTTCTTTGCCT3′ (-1574/-1555 nt).

An unlabeled 20 bp dsoligo spanning this run of Ts in site A (-3469/-3450 nt) competed with the *NP*/*NMP4* binding to the labeled 53 bp

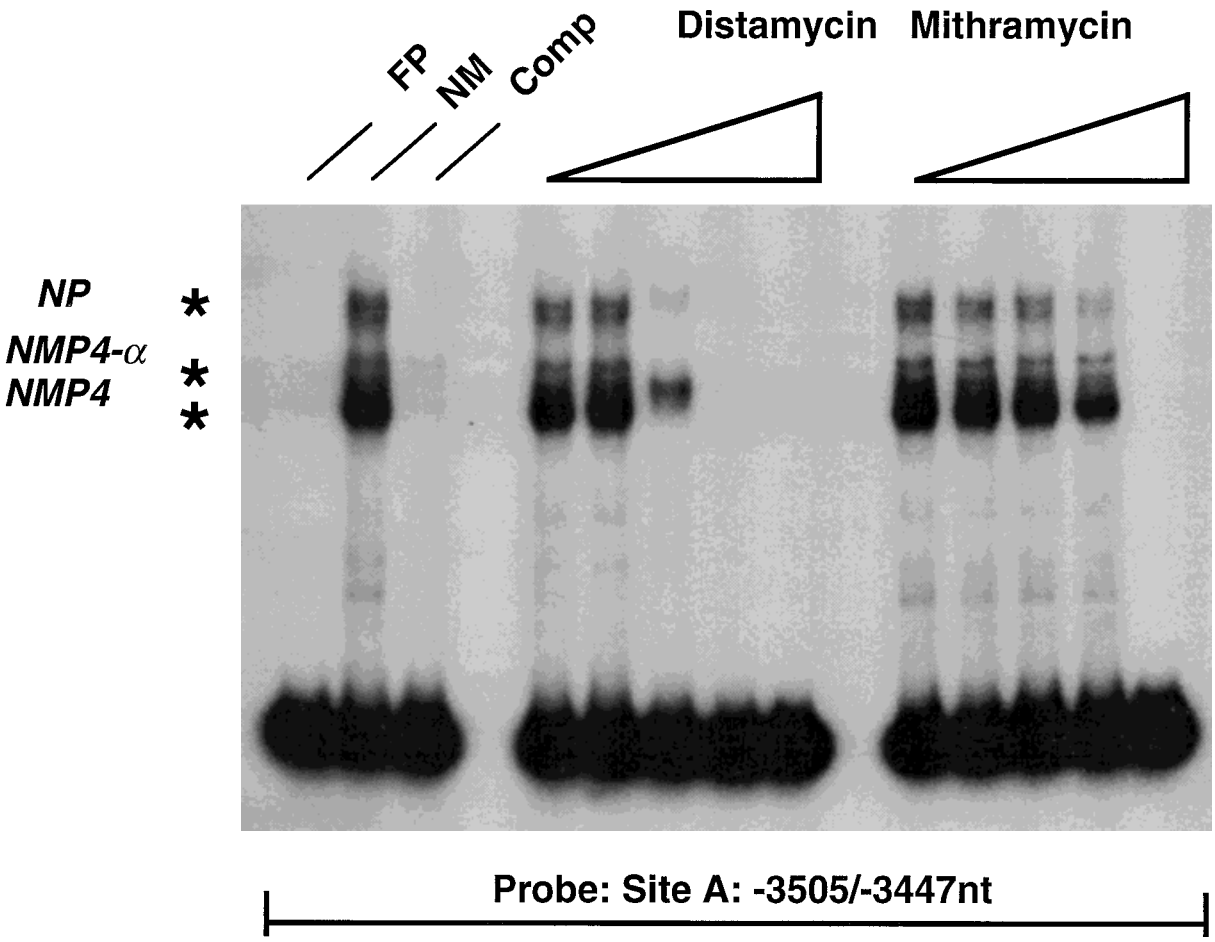

Fig. 8. Drugs that bind to the minor groove of AT-rich DNA compete with *NMP4* binding to the poly(dT) regions of the COL1A1 promoter. FP, free probe; NM, nuclear matrix extracts from UMR 106-01 cells (2 μg/lane); Comp, specific competitor (unlabeled site A, 100 M excess). Labeled probe, site A (-3489/-3434 nt). Distamycin is a drug that binds to the minor groove of AT-rich DNA (concentration from 1.5–100 μM; the last lane is free probe + 100 μM drug). Mithramycin A is a drug that binds to the minor groove of GC-rich DNA (concentrations as with distamycin). Similar response was observed using site B as a labeled probe.

fragment (-1594/-1541 nt, site B) (Fig. 3) and to the labeled 55 bp fragment (-3489/-3434 nt, site A) (data not shown). This unlabeled 20 bp dsoligo also competed *NMP4* binding to the 112 bp COL1A1 fragment (-3518/-3406 nt) (Fig. 4). However, *NP/NMP4* did not bind to any T-rich dsoligo since an oligo to a portion of the T-rich, β-casein element (BCE-1) [Schmidhauser et al., 1992] did not compete for *NP/NMP4* binding activity along site B (Fig. 3) or site A (data not shown). Consistent with these competition results, the 20 bp labeled oligos spanning -3469/-3450 nt (site A) and -1574/-1555 nt (site B) recovered *NP/NMP4* binding activities from osteoblast-like cell nuclear matrix preparations (Fig. 4). Interestingly, the single-stranded, T-rich, sense COL1A1 promoter fragments between -3469/-3450 nt and -1574/-1555 nt recovered the *NP/NMP4* binding, but the antisense, A-rich strands did not (Fig. 4).

Data from methidium propyl EDTA (MPE) footprinting, using the 112 bp probe (-3518/-3406 nt) indicated protection between -3468/-3448 nt (site A) over the T-rich region (Fig 5), concordant with our EMSA results. We observed that site A exhibited some protection from MPE cleavage in the absence of nuclear matrix protein (Fig. 5) indicative of a narrow DNA minor groove. Mutational analysis demonstrated that mutating two Ts (-1567 and -1565 nt) to Gs in the run of nine thymidines nearly abolished *NMP4* and *NP* binding to site B (Fig. 6). Interestingly, mutating these two Ts with a G and an A did not completely abrogate bind-

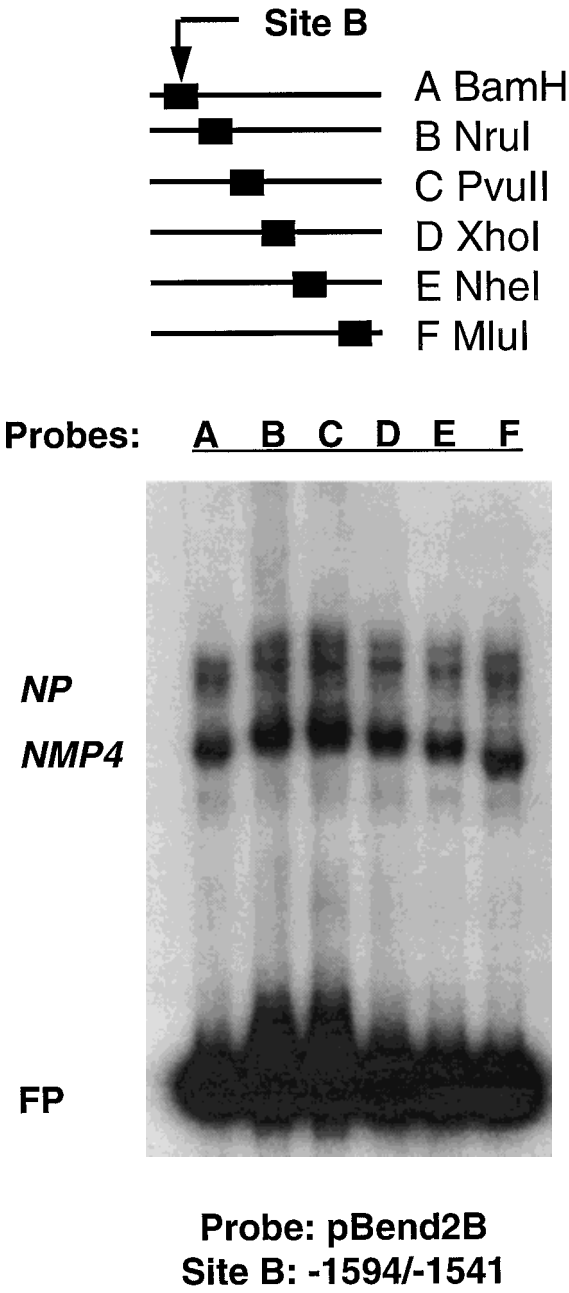

Fig. 9. Circular permutation analysis demonstrating *NP/NMP4*-induced bending of the rat COL1A1 promoter fragment at site B. The plasmid pBend2 [Kim et al., 1989] was used to generate several DNA fragments of identical length and nucleotide sequence in which the protein-binding nucleotide sequence (site B, -1594/-1541 nt) was located in circular permutations. The anomalous migration of both *NP* and *NMP4* bands indicates that these proteins bend the DNA fragment. Note that the free probes (no protein binding) all migrate with equal mobility. Using the equation $\mu_M/\mu_E = \cos(\alpha/2)$, we determined that the DNA flexure angle α was ~47°.

ing. Similar results were obtained using mutations of site A as our labeled probes. We speculate that the disruption of the thymidine sequence with more than one guanine nucleotide mutation may alter the structure of the binding element, such as its very narrow minor groove. However, replacing a T with an A may preserve the structure of the narrow minor groove.

NMP4 Was Recovered in Nuclear Matrix Extracts From Rat Bone

Although we have previously recovered *NMP4* binding activity along the rat COL1A1 promoter using nuclear matrix extracts derived from cultured rat primary spongiosa osteoblasts [Alvarez et al., 1997], it was not clear whether this protein was present in bone tissue. EMSA of nuclear matrix extracts derived from the primaryspongiosa of the metaphyseal femurs of young male rats exhibited *NMP4* binding activity along the COL1A1 promoter, in addition to high mobility protein-DNA bands (Fig. 7). Interestingly, we did not observe any *NP* binding activity in these in vivo nuclear matrix fractions, although since this protein is primarily localized to the soluble nuclear fraction in cultured bone cells we cannot yet conclude that it is absent in the primary spongiosa of rat bone.

NP/NMP4 Exhibit Characteristics of Architectural Transcription Factors

Architectural transcription factors often recognize structural anomalies of the DNA, such as a very narrow minor groove formed by runs of As and Ts [Churchill and Travers, 1991]. Both distamycin and berenil, drugs that bind to the minor groove of DNA [Abu-Daya et al., 1995], competed with *NP/NMP4* binding to sites A and B of theCOL1A1 promoter (Fig. 8). Mithramycin, a drug that binds to the minor groove of GC-rich DNA, was a weaker competitor (Fig. 8). Interestingly, the binding of distamycin to the minor groove of site A also interfered with the interaction of *NMP4*-α and *NP* proteins at this site. Circular permutation analysis indicated that *NP/NMP4* binding to the rat COL1A1 promoter induced a bend in the DNA (Fig. 9). Both the *NP*- and *NMP4*-COL1A1[site B] complexes exhibited a position-dependent mobility (Fig. 9). Nuclear matrix extracts from rat bone exhibited a similar anomalous electrophoretic mobility (data not

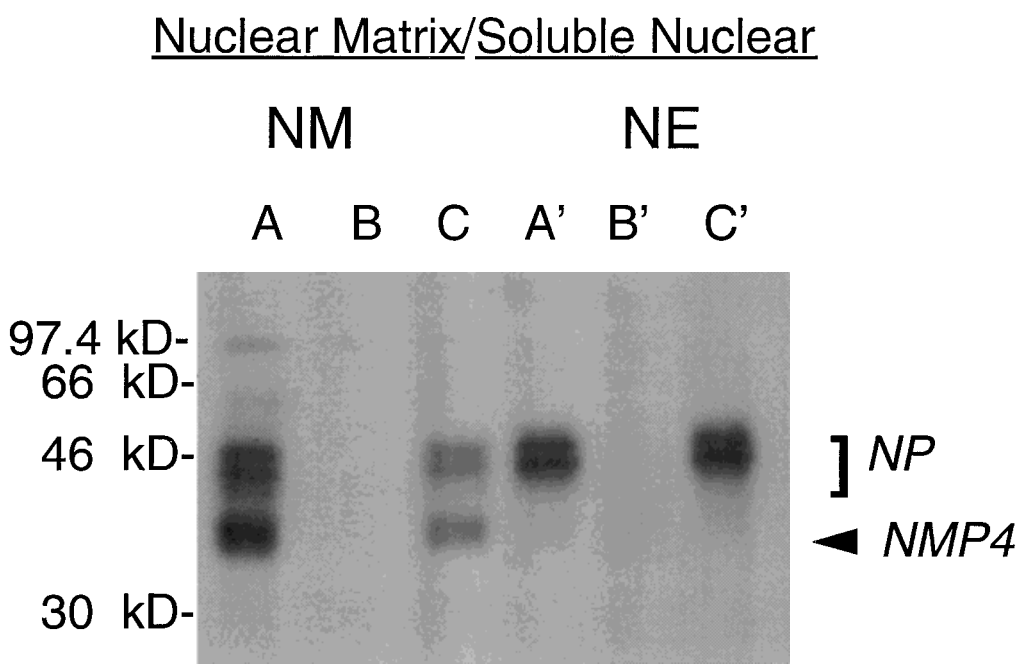

Fig. 10. UV cross-linking of *NP* and *NMP4* with COL1A1 site B (-1594/-1541 nt). Probe, BrdU-substituted site B, labeled with α-^{32}PdCTP; NM, nuclear matrix extract from UMR 106-01 cells; NE, soluble nuclear extracts. **Lanes A/A':** 2 µg of NM/NE protein cross-linked to probe. **Lanes B/B':** Proteins/probe plus unlabeled specific competitor (site B, 100 M excess). **Lanes C/C':** Proteins/probe plus unlabeled nonspecific competitor (NMP-2/Cbfa-1 [Banerjee et al., 1996], 100 M excess). Controls including binding reactions that were not exposed to UV light and cross-linked extracts incubated with proteinase K exhibited no signals (data not shown).

shown). The *NP*- and *NMP4*-induced DNA flexure angle a was determined to be ~47_ using the equation $\mu_M/\mu_E = \cos(\alpha/2)$.

UV Cross-Linking Indicates That the *NMP4* Complex Has a Molecular Weight of ~40 kD and the NP Doublets Molecular Weights of ~46 kD and 43 kD

To determine the molecular weight of the complexes we have designated *NMP4* and *NP*, we performed UV cross-linking using the COL1A1 promoter fragments containing sites A (-3489/-3434 nt) and B (-1594/ -1541 nt) (Fig. 10). *NMP4* was determined to have a molecular weight of ~40 kD using either of the poly(dT) COL1A1 sites A or B as probes. This DNA binding activity appears to consist of two closely spaced bands. The doublet bands designated by us as *NP* had molecular weights of ~46 kD and ~43 kD. Similar protein-DNA binding patterns were observed in Southwestern blots of the nuclear matrix and soluble nuclear matrix fractions using site B (-1574/-1555 nt) as a probe (data not shown).

Parathyroid Hormone Modulates the DNA-Binding Activity of *NP/NMP4*, ROS 17/2.8 Cells

Treatment of ROS 17/2.8 cells with rat PTH(1-34) [rPTH(1-34)] increased *NMP4* binding activity along both sites A and B of the COL1A1 promoter by approximately two- to fourfold as determined by densitometry analysis (Fig. 11). Cells were challenged with 10 nM rPTH(1-34) or the same volume of vehicle (10 mM acetic acid) for 72 h. Similar results were observed with the UMR 106-01 cells. There was a concomitant decrease in COL1A1 mRNA expression in the PTH-treated cells.

DISCUSSION

The capacity of *NP/NMP4* to bend the COL1A1 promoter DNA upon binding within the minor groove of the two homologous poly(dT) sites is characteristic of architectural transcription factors, although the functional significance of *NP/NMP4* remains to be determined. These kinds of trans-acting proteins often recognize local structural anomalies (i.e., a narrow

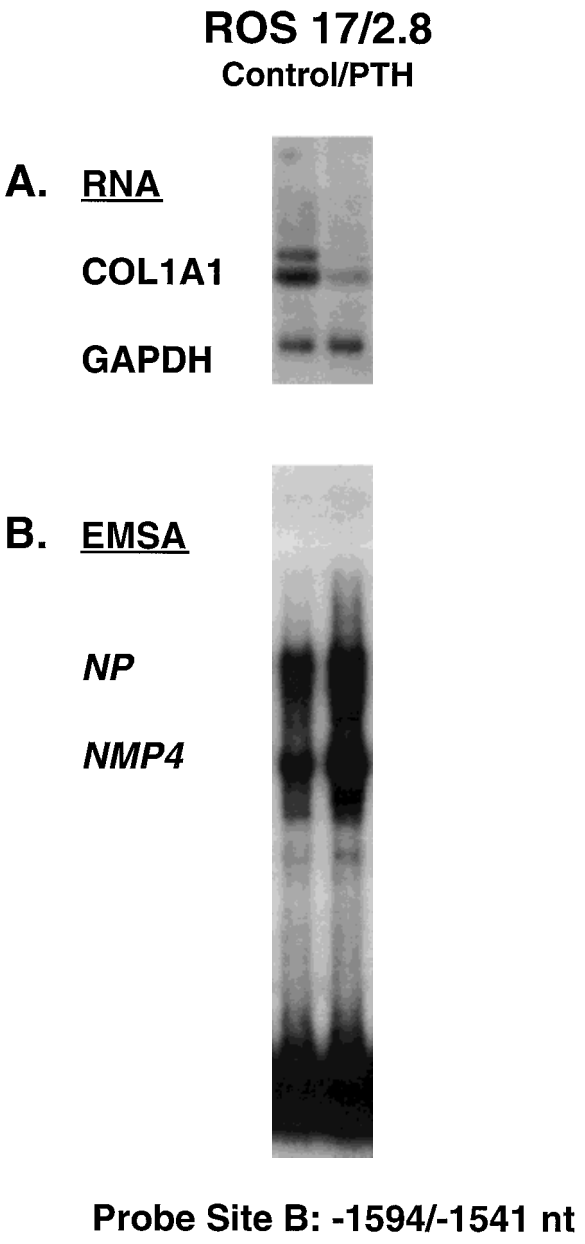

Fig. 11. Parathyroid hormone modulates the DNA binding activity of *NMP4* at the poly(dT) sites of the COL1A1 promoter. **A:** Northern analysis of COL1A1 and GAPDH expression in ROS 17/2.8 cells treated with rPTH(1-34) (10 nM, 72 h) or vehicle (control). **B:** EMSA analysis of PTH-modulated *NP/NMP4* binding activity along the rat COL1A1 promoter. Labeled probe, site B (-1594/-1541 nt); protein, nuclear matrix extracts from ROS 17/2.8 cells, 2 µg/lane. Cells treated as described in A. Exposure to PTH increased *NP/NMP4* DNA binding activity and decreased COL1A1 mRNA expression. These results are representative of several experiments.

minor groove) conferred by AT-rich DNA on the B helix [Churchill and Travers, 1991]. Therefore, the two homologous poly(dT) sites likely comprise a consensus context [Churchill and Travers, 1991] rather than a consensus sequence, and the sensitivity of *NP/NMP4* binding to nucleotide mutations in these sites may derive more from a structural derangement of the minor groove.

NP/NMP4 binding to single-stranded DNA is typical of many major nuclear matrix proteins, including the lamins B and C, the matrins, and nucleolin [Dickinson and Kohwi-Shigematsu, 1995; Hakes and Berezney, 1991]. The binding preference of *NP/NMP4* to the T-rich strand over the A-rich strand is similar to the yeast DNA-binding proteins PUB1, PAB1 [Cockell et al., 1994] and ssARS-T [Schmidt et al., 1991], putatively involved in replication initiation in yeast ARS elements.

Nuclear matrix architectural transcription factors, as an ostensible class of trans-acting proteins, would be particularly suited for coupling cell structure and promoter geometry. Nuclear DNA is physically linked to the cell's substructure, or tissue matrix [Maniotis et al., 1997], in part by the nuclear matrix proteins that mediate the formation of the topological and functionally distinct DNA loop domains in the chromatin [Davie, 1995; Pienta et al., 1991; Mirkovitch et al., 1984]. These DNA loops are fastened to the nuclear matrix–chromosome scaffold through matrix-attachment regions (MARs), typically AT-rich regions of DNA, 200–800 base pairs in length, that exhibit characteristics of chromatin boundary elements [Davie, 1995; Boulikas, 1995; Pienta et al., 1991; Phi-Van et al., 1990; Mirkovitch et al., 1984].

MAR-binding nuclear matrix proteins not only couple the DNA to the cell substructure but participate in transcription [Herrscher et al., 1995; Dickinson and Kohwi-Shigematsu, 1995; Forrester et al., 1994; Nakagomi et al., 1994]. The nuclear matrix proteins SATB1 (special AT-rich sequence-binding protein) [Nakagomi et al., 1994], and Bright (B-cell regulator of IgH transcription) [Herrscher et al., 1995; Forrester et al., 1994] recognize the minor groove of specific MAR sites and putatively regulate gene expression by mediating the relief of negative superhelical strains through the stabilization of the base unpairing of these regions [Dickinson and Kohwi-Shigematsu, 1995;

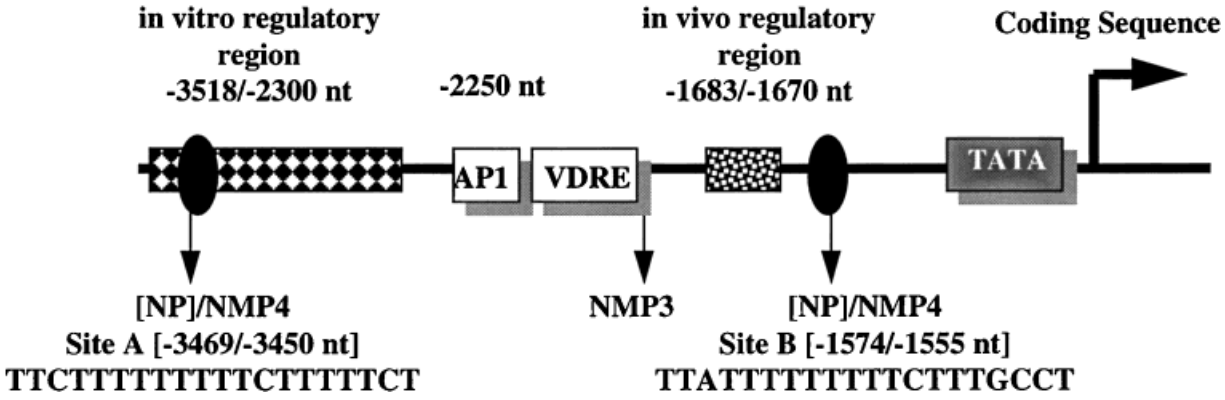

Fig. 12. Schematic representation of rat COL1A1 promoter showing the nuclear matrix binding sites. [NP] is shown in brackets to indicate that this soluble nuclear protein has yet to be recovered in vivo. Not drawn to scale.

Bode et al., 1992; Kohwi-Shigematsu and Kohwi, 1990].

Architectural transcription factors are similar to MAR-binding nuclear matrix proteins in their propensity for binding within the minor groove of AT-rich DNA and mediating changes in gene activity by altering DNA structure. Architectural trans-acting proteins often lack distinct activation domains and do not necessarily initiate transcription themselves or even interact with the other regulatory proteins [Wolffe, 1994; Grosschedl et al., 1994; Van der Vliet and Verrijzer, 1993]. Like the MAR-binding nuclear matrix proteins, they may be proximal or distal to the target regulatory region. LEF-1 binds to the T-cell receptor α-gene enhancer and induces a sharp bend in the DNA, bringing together two nonadjacent cis-regulatory sites without interacting with these other transcription factors [Giese et al., 1992, 1995; Grosschedl et al., 1994]. Therefore, a nuclear matrix architectural transcription factor could bind directly to the promoter, outside the confines of the MAR, or other cis-regulatory elements, and render changes in tissue-matrix organization into alterations in promoter conformation.

NMP4, as a PTH-responsive nuclear matrix architectural transcription factor, could elucidate some exceptional aspects of osteoblast COL1A1 expression (see Fig. 12). The putative changes in microarchitecture that mediate the shift in the promoter cis-regulatory elements between osteoblasts in tissue and those in vitro may be a consequence of distinct promoter geometries as governed by *NP/NMP4* binding. Although *NMP4* was observed in extracts from both cell lines and bone tissue, *NP* binding activity was only recovered in the in vitro preparations. Additionally, high-mobility COL1A1-binding nuclear matrix proteins were recovered from bone but not the cell lines. This may indicate distinct higher-order nucleoprotein complexes along the COL1A1 promoter in bone and cell culture.

PTH-induced changes in osteoblast cyto- and nuclear architecture may culminate in *NMP4*-mediated alterations in COL1A1 promoter geometry. PTH-induced a retraction of the osteoblast lamellipodia, a massive reorganization of microfilaments, and a decrease in the number of stress fibers [Egan et al., 1991; Aubin et al., 1983]. Acute and chronic exposure to hormone resulted in transient and stable changes in osteoblast cytoskeletal architecture, respectively [Lormi and Marie, 1990; Egan et al., 1991; Bidwell et al., 1996]. NuMA, a component of the nuclear matrix [Compton and Cleveland, 1994; Zeng et al., 1994], was upregulated by PTH in ROS 17/2.8 cells [Bidwell et al., 1996; Torrungruang et al., 1998]. Therefore, a mechanical pathway by which PTH effects a change in COL1A1 promoter geometry may include a microfilament-NuMA-*NMP4* network.

The *NMP4*-COL1A1 interactions may underlie in part the apparent paradoxical regulation of collagen synthesis by PTH in vivo and in vitro. Low, intermittent doses of PTH resulted in a net increase in bone mass in both experimental animals and humans, including an upregulation of COL1A1 synthesis [Dobnig and Turner, 1995; Schmidt et al., 1995; Whitfield and Morley, 1995; Dempster et al., 1993]. However, PTH typically attenuated COL1A1 expression in most in vitro osteoblast preparations [Kream et al., 1993; Partridge et al., 1989].

Differences in COL1A1 promoter geometry, as mediated by the PTH-responsive *NMP4* nuclear matrix protein, may contribute to these opposing responses to this hormone.

The molecular consequence of altering cell structure may be the twisting or bending of those promoters coupled to the tissue matrix. The structural continuum from the extracellular matrix to the geometry of the promoter may directly link osteoblast deformation with a realignment of those genes coupled to the tissue matrix and tug genes into an altered state of activity. This kind of mechanism may be particularly significant to connective tissue where hormone- and mechanical load–induced changes in gene expression often involve a modulation of cell architecture.

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REFERENCES

Abu-Daya A, Brown PM, Fox KR (1995): DNA sequence preference of several AT-selective minor groove binding ligands. Nucleic Acids Res 23:3385–3392.

Ali NN, Melhuish PB, Boyde A, Bennet A, Jones SJ (1990): Parathyroid hormone, but not prostaglandin E2, changes the shape of osteoblasts maintained on bone in vitro. J Bone Miner Res 5:115–121.

Alvarez M, Onyia J, Hock J, Long H, Xu W, Bidwell J (1997): Rat osteoblast and osteosarcoma nuclear matrix proteins bind with sequence specificity to the rat type I collagen promoter. Endocrinology 138:482–489.

Aubin JE, Alders E, Heersche JMN (1983): A primary role for microfilaments but not microtubules in hormone induced cytoplasmic retraction. Exp Cell Res 143:439–450.

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl D (eds) (1987): "Current Protocols in Molecular Biology." New York: John Wiley & Sons, Inc.

Banerjee C, Hiebert SW, Stein JL, Lian JB, Stein GS (1996): An AML-1 consensus sequence binds an osteoblast-specific complex and transcriptionally activates the osteocalcin gene. Proc Natl Acad Sci U S A 93:4968–4973.

Bedalov A, Salvatori R, Dodig M, Kronenberg MS, Kapural B, Bogdanovic Z, Kream BE, Woody CO, Clark SH, Mack K, Rowe DW, Lichtler AC (1995): Regulation of COL1A1 expression in type I collagen producing tissues: Identifcation of a 49 base pair region which is required for transgene expression in bone of transgenic mice. J Bone Miner Res 10:1443–1451.

Benya PD (1988): Modulation and reexpression of the chondrocyte phenotype; mediation by cell shape and microfilament modification. Pathology and Immunopathology Research 7:51–54.

Benya PD, Shaffer JD (1982): Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. Cell 30:215–224.

Bidwell J, Feister H, Swartz D, Onyia J, Holden J, Hock J (1996): Parathyroid hormone regulates the expression of rat osteoblast and osteosarcoma nuclear matrix proteins. J Cell Biochem 63:374–383.

Bode J, Kohwi Y, Dickinson L, Joh T, Klehr K, Mielke C, Kohwi-Shigematsu T (1992): Biological significance of unwinding capability of nuclear matrix–associating DNAs. Science 255:195–197.

Bogdanovic Z, Bedalov A, Krebsbach PH, Pavlin D, Woody CO, Clark SH, Thomas HF, Rowe DW, Kream BE, Lichtler AC (1994): Upstream regulatory elements necessary for expression of the rat COL1A1 promoter in transgenic mice. J Bone Miner Res 9:285–292.

Boulikas T (1995): Chromatin domains and prediction of MAR sequences. Int Rev Cytol 162A:279–388.

Bronner F, Stein WD (1992): Modulation of bone calcium-binding sites regulates plasma calcium: An hypothesis. Calcif Tissue Int 50:483–489.

Churchill MEA, Travers AA (1991): Protein motifs that recognize structural features of DNA. Trends Biochem Sci 16:92–97.

Cockell M, Frutiger S, Hughes GJ, Gasser SM (1994): The yeast protein encoded by PUB1 binds T-rich single stranded DNA. Nucleic Acids Res 22:32–40.

Compton DA, Cleveland DW (1994): NuMA, a nuclear protein involved in mitosis and nuclear reformation. Current Opin Cell Biol 6:343–346.

Davie JR (1995): The nuclear matrix and the regulation of chromatin organization and function. Int Rev Cytol 162A: 191–250.

Dempster DW, Cosman F, Parisien M, Shen V, Lindsay R (1993): Anabolic actions of parathyroid hormone on bone. Endocr Rev 14:690–709.

Dhawan J, Lichtler AC, Rowe DW, Farmer SR (1991): Cell adhesion regulates pro-a1(I) collagen mRNA stability and transcription in mouse fibroblasts. J Biol Chem 266:8470–8475.

Dickinson LA, Kohwi-Shigematsu T (1995): Nucleolin is a matrix-attachment region DNA-binding protein that specifically recognizes a region with high base-unpairing potential. Mol Cell Biol 15:456–465.

Diegelmann RF, Peterkofsky B (1972): Inhibition of collagen secretion from bone and cultured fibroblasts by microtubule disruptive drugs. Proc Natl Acad Sci U S A 69:892–896.

Dignam JD, Lebovitz RM, Roeder RG (1983): Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res 11:1475–1489.

Dobnig H, Turner R (1995): Evidence that intermittent treatment with parathyroid hormone increases bone formation in adult rats by activation of bone lining cells. Endocrinology 136:3632–3638.

Dodig M, Kronenberg MS, Bedalov A, Kream BE, Gronowicz G, Clark SH, Mack K, Liu YH, Maxon R, Pan ZZ, Upholt WB, Rowe DW, Lichtler AC (1996): Identification of a TAAT-containing motif required for high level expression of the COL1A1 promoter in differentiated osteoblasts of transgenic mice. J Biol Chem 271:16422–16429.

Egan JJ, Gronowicz G, Rodan G (1991): Parathyroid hormone promotes the disassembly of cytoskeletal actin and myosin in cultured osteoblastic cells: Mediation by cyclic AMP. J Cell Biochem 45:101–111.

Ferrari S, Harley VR, Pontiggia A, Goodfellow PN, Lovell-Badge R, Bianchi ME (1992): SRY, like HMG1, recognizes sharp angles in DNA. EMBO J 11:4497–4506.

Ferrier J, Ward-Kesthely A, Heersche JN, Aubin JE (1988): Membrane potential changes, cAMP stimulation and contraction in osteoblast-like UMR 106 cells in response to calcitonin and parathyroid hormone. Bone Miner 4:133–145.

Forrester WC, Genderen Cv, Jenuwein T, Grosschedl R (1994): Dependence of enhancer-mediated transcription of the immunoglobulin μ gene on nuclear matrix attachment regions. Science 265:1221–1225.

Giese K, Cox J, Grosschedl R (1992): The HMG domain of lymphoid enhancer factor 1 bends DNA and facilitates assembly of functional nucleoprotein structures. Cell 69: 185–195.

Giese K, Kingsley C, Kirshner JR, Grosschedl R (1995): Assembly and function of a TCRa enhancer complex is dependent on LEF-1–induced DNA bending and multiple protein-protein interactions. Genes Dev 9:995–1008.

Grosschedl R, Giese K, Pagel J (1994): HMG domain proteins: Architectural elements in the assembly of nucleoprotein structures. Trends Genet 10:94–100.

Hakes DJ, Berezney R (1991): DNA binding properties of the nuclear matrix and individual nuclear matrix proteins. Evidence for salt-resistant DNA binding sites. J Biol Chem 266:11131–11140.

Herrscher RF, Kaplan MH, Lesz DL, Das C Scheuermann R, Tucker PW (1995): The immunoglobulin heavy-chain matrix-associating regions are bound by Bright: a B cell–specific trans-activator that describes a new DNA-binding protein family. Genes Dev 9:3067–3082.

Hertzberg RP, Dervan PB (1984): Cleavage of DNA with Methidiumpropyl-EDTA-Iron(II): Reaction conditions and product analyses. Biochemistry 23:3934–3945.

Hofmann JFX, Gasser SM (1991): Identification and purification of a protein that binds the yeast ARS consensus sequence. Cell 64:951–960.

Kerppola TK, Curran T (1993): Selective DNA bending by a variety of bZIP proteins. Mol Cell Biol 13:5479–5489.

Kim J, Zwieb C, Wu C, Adhya S (1989): Bending of DNA by gene-regulatory proteins: Construction and use of a DNA bending vector. Gene 85:15–23.

Kohwi-Shigematsu T, Kohwi Y (1990): Torsional stress stabilizes extended base unpairing in suppressor sites flanking immunoglobulin heavy chain enhancer. Biochemistry 29:9551–9560.

Kream BE, LaFrancis D, Petersen DN, Woody C, Clark S, Rowe DW, Lichtler A (1993): Parathyroid hormone represses a1(I) collagen promoter activity in cultured calvariae from neonatal transgenic mice. Mol Endocrinol 7:399–408.

Lormi A, Marie PJ (1990): Changes in cytoskeletal proteins in response to parathyroid hormone and 1,25-dihydroxyvitamin D in human osteoblastic cells. Bone Miner 10: 1–12.

Maniotis AJ, Chen CS, Ingber DE (1997): Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. Proc Natl Acad Sci U S A 94:849–854.

Mirkovitch J, Mirault M-E, Laemmli UK (1984): Organization of the higher order chromatin loop: Specific DNA attachment sites on nuclear scaffold. Cell 39:223–232.

Nakagomi K, Kohwi Y, Dickinson LA, Kohwi-Shigematsu T (1994): A novel DNA-binding motif in the nuclear matrix attachment DNA-binding protein SATB1. Mol Cell Biol 14:1852–1860.

Nickerson JA, Blencowe BJ, Penman S (1995): The architectural organization of nuclear metabolism. Int Rev Cytol 162A:67–123.

Partridge NC, Dickson CA, Kopp K, Teitelbaum SL, Crouch EC, Kahn AJ (1989): Parathyroid hormone inhibits collagen synthesis at both ribonucleic acid and protein levels in rat osteogenic sarcoma cells. Mol Endocrinol 3:232–239.

Phi-Van L, von Kries JP, Ostertag W, Strätling WH (1990): The chicken lysozyme 5' matrix attachment region increases transcription from a heterologous promoter in heterologous cells and dampens position effects on the expression of transfected genes. Mol Cell Biol 10:2302–2307.

Pienta KJ, Hoover CN (1994): Coupling of cell structure to cell metabolism and function. J Cell Biochem 55:16–21.

Pienta KJ, Getzenberg RH, Coffey DH (1991): Cell structure and DNA organization. Eukaryotic Gene Expression 1:355–385.

Scherft JP, Heersche JNM (1975): Accumulation of collagen containing vacuoles in osteoblasts after administration of colchicine. Cell Tissue Res 157:353–365.

Schmidhauser C, Casperson GF, Myers CA, Sanzo KT, Bolten S, Bissel MJ (1992): A novel transcriptional enhancer is involved in the prolactin- and extracellular matrix–dependent regulation of β-casein gene expression. Mol Biol Cell 3:699–709.

Schmidt AM, Herterich SU, Krauss G (1991): A single-stranded DNA binding protein from S. cerevisiae specifically recognizes the T-rich strand of the core sequence of ARS elements and discriminates against mutant sequences. EMBO J 10:981–985.

Schmidt IU, Dobnig H, Turner RT (1995): Intermittent parathyroid hormone treatment increases osteoblast number, steady state messenger ribonucleic acid levels for osteocalcin, and bone formaiton in tibial metaphysis of hypophysectomized female rats. Endocrinology 136: 5127–34.

Thompson JF, Landy A (1988): Empirical estimation of protein-induced DNA bending angles: Applications to lambda site-specific recombination complexes. Nucleic Acids Res 16:9687–9705.

Torrungruang K, Feister F, Swartz D, Hancock EB, Hock J, Bidwell JP (1998): Parathyroid hormone (PTH) regulates the expression of the nuclear mitotic apparatus protein (NuMA) in the osteoblast-like cells, ROS 17/2.8. Bone (in press).

van der Vliet PC, Verrijzer CP (1993): Bending of DNA by transcription factors. Bioessays 15:25–32.

Verrijzer CP, van Oosterhout JAWM, van Weperen WW, van der Vliet PC (1991): POU proteins bend DNA via the POU-specific domain. EMBO J 10:3007–3014.

Whitfield JF, Morley P (1995): Small bone-building fragments of parathyroid hormone: New therapeutic agents for osteoporosis. Trends Pharmacol Sci 16:382–386.

Wolffe AP (1994): Architectural transcription factors. Science 264:1100–1101.

Wu HM, Crothers DM (1984): The locus of sequence-directed and protein-induced DNA bending. Nature 308: 509–513.

Zeng C, He D, Brinkley BR (1994): Localization of NuMA protein isoforms in the nuclear matrix of mammalian cells. Cell Motil Cytoskeleton 29:167–176.