DNase I Hypersensitive Sites in Promoter Elements Associated with Basal and Vitamin D Dependent Transcription of the Bone-Specific Osteocalcin Gene†

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ABSTRACT: Nuclease hypersensitive sites were mapped in the proximal promoter of the osteocalcin gene, which is expressed only in bone cells exhibiting the mature osteoblast phenotype. Nuclei from proliferating and confluent rat osteosarcoma (ROS) 17/2.8 cells were subjected to DNase I digestion, and hypersensitivity was assayed by the indirect end-labeling method, using osteocalcin gene probes. Hypersensitive sites were detected in two promoter domains: −590 to −390, which spans the vitamin D responsive element, and −170 to −70, which spans the TATA box and the CCAAT-containing OC box domain. Together, these elements regulate basal and vitamin D enhanced osteocalcin gene transcription. We observed a parallel relationship between the intensity of bands representing the hypersensitive sites and the extent to which the osteocalcin gene is transcribed. Both in confluent cultures and in response to vitamin D, when osteocalcin transcription was upregulated, the hypersensitive bands were significantly intensified. Additionally, the bands were decreased under conditions that downregulate osteocalcin gene transcription. A functional relationship between the presence of hypersensitive sites and osteocalcin gene transcription is further supported by the absence of hypersensitivity in nonosseous cells that do not express osteocalcin, although these proliferating cells exhibited hypersensitivity in a cell cycle regulated histone gene promoter. Our results suggest the involvement of chromatin structure in transcriptional responsiveness of the osteocalcin gene to physiologic modulation.

The osteocalcin gene encodes a 10-kDa bone-specific protein, which is expressed only postproliferatively during the differentiation of normal diploid osteoblasts, when mineralization of the extracellular matrix is occurring (Aronow et al., 1990; Owen et al., 1990a). In transformed bone cells, e.g., ROS 17/2.8 osteosarcoma cells, the osteocalcin gene is expressed continually, reaching its highest level when the cells become confluent (Bortell et al., 1993). In normal diploid rat osteoblasts, as well as in ROS 17/2.8 cells, the osteocalcin gene is responsive to regulation by steroid hormones (glucocorticoids and vitamin D) only when basal expression is ongoing (Lian et al., 1991; Owen et al., 1991, 1993; Shalhoub et al., 1992). The rat osteocalcin gene promoter is composed of a modularly organized series of regulatory elements, which independently and in combination contribute to gene transcription (Bortell et al., 1992, 1993; Markose et al., 1990; Owen et al., 1990b, 1993). These include the TATA box (−44 to −31), which is both contiguous to and overlapped by glucocorticoid responsive elements (GRE's) (Heinrichs et al., 1993a; Morrison et al., 1989; Stromstedt et al., 1992); the OC box (−113 to −76), which contains a central CCAAT motif and has been shown to control, in part, the basal level of transcription of this gene (Bortell et al., 1993; Heinrichs et al., 1993b; Lian et al., 1991; Markose et al., 1990); and the vitamin D responsive element (VDRE, −466 to −437), which provides a key component of steroid hormone mediated transcriptional enhancement (Bortell et al., 1992; Demay et al., 1990; Markose et al., 1990; Owen et al., 1991; Terpening et al., 1991). The presence of AP-1 sites within the OC box and VDRE domains of the osteocalcin gene promoter provides the potential for mutually exclusive occupancy of basal regulatory and enhancer elements by sequence-specific factors and fos–jun complexes contributing to transcriptional control (Markose et al., 1990; Owen et al., 1990b). Additionally, evidence for synergism of activities at the VDRE, GRE, and TATA domains has been reported (Bortell et al., 1992; Morrison et al., 1989). We have also recently found that sequences residing within the +39 to +104 segment function in vivo as a transcriptional inhibitory sequence (Frenkel et al., 1993).

Analysis of chromatin structure indicates that active or potentially active genes are typically marked by the presence of nuclease sensitive domains. These regions have been suggested to reflect the absence of a classical nucleosome structure and the binding of specific non-histone chromosomal proteins (Elgin, 1988, 1990; Felsenfeld, 1992; Gross & Garrard, 1988). Hypersensitivity to DNase I has been used successfully to show changes in chromatin structure associated with transcriptional activation of heat shock inducible genes (Cartright & Elgin, 1986; Keene et al., 1981; Wu, 1980), steroid hormone regulated genes (Hager et al., 1980; Kaye et al., 1986; Zaret & Yamamoto, 1984), and cell cycle regulated genes (Chrysogelos et al., 1989; Moreno et al., 1986) and the onset of expression of specific genes during differentiation (Mollers et al., 1992). We have taken this approach to analyze the presence of hypersensitive sites in the osteocalcin gene in ROS 17/2.8 cells where the gene is continually expressed. Additionally, we have studied the effect of vitamin D and the synthetic glucocorticoid dexamethasone on the hypersensitive site pattern in both proliferating and confluent cell cultures expressing low and high levels of osteocalcin.

Here, we report that DNase I hypersensitive sites are evident at the basal regulatory OC box and TATA/GRE elements, as well as flanking the VDRE, in osteosarcoma cells actively transcribing the osteocalcin gene. DNase I hypersensitivity is not observed at these principal osteocalcin gene transcrip-

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tional regulatory elements in nonosseous cells that do not express osteocalcin. However, vitamin D enhancement of osteocalcin gene transcription is accompanied by increased nuclease sensitivity at the VDRE and the OC box. These results suggest that both activation and steroid hormone enhancement of osteocalcin gene transcription involve a functional relationship between changes in chromatin structure and sequence-specific interactions of transactivation factors.

MATERIALS AND METHODS

Cell Culture. Rat osteosarcoma-derived ROS 17/2.8 cells (a gift from S. Rodan and G. Rodan, Merck, Sharp, and Dohme, West Point, PA) were maintained as described (Majeska et al., 1980). Cells were treated with 10^{-8} M 1,25-dihydroxyvitamin D_3 or vehicle for 4 or 24 h, or with 10^{-7} M dexamethasone where designated. Osteocalcin protein was quantitated in the cell culture media by radioimmunoassay (Gundberg et al., 1984). Rat hepatoma-derived H4 cells (a gift from S. Grimes) were maintained as previously described (Wolfe et al., 1989).

DNase I Hypersensitivity Studies. DNase I hypersensitivity analyses were performed according to the indirect end-labeling method (Wu, 1980). Nuclei were isolated from ROS 17/2.8 cells on days 3 (proliferating) and 8 (confluent) or from confluent rat hepatoma cell cultures and partially digested with DNase I (Worthington Biochemicals, Freehold, NJ) for 10 min at 20 °C, as described (Moreno et al., 1986). For osteocalcin gene analysis, DNA was isolated and completely digested with the restriction endonucleases *Bam*HI or *Apa*I (New England Biolabs, Beverly, MA). For histone H4 gene studies, DNA was digested with *Hin*dIII. Digested DNA was resolved in 1% or 2% agarose gels and transferred to a nylon membrane (Zeta-Probe, Bio-Rad Laboratories, Melville, NY), following the manufacturer's recommendations. Probes were prepared by restriction endonuclease digestion of the osteocalcin gene clone pOC3.4, containing the rat osteocalcin gene and flanking sequences, and PJA5, which includes the rat somatic histone H4 gene (Wolfe et al., 1989), and labeled with [α-^{32}P]dCTP by nick translation using reagents from Boehringer Mannheim (Indianapolis, IN). *Hin*dIII-digested bacteriophage λ DNA and *Hin*fI-digested pBR322 DNA, labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP, were used as sized DNA markers.

DNase I Digestion of ROS 17/2.8 Genomic DNA. Purified genomic DNA from ROS 17/2.8 cells (400 μg/mL) was incubated at 20 °C for 2 min with increasing amounts of DNase I. The reaction was stopped by addition of a stock solution to a final concentration of 25 mM EDTA, pH 8.0, 0.5% (w/v) SDS, and 100 μg/mL proteinase K, and the mixture was incubated at 37 °C, which was followed by a phenol/chloroform extraction and ethanol precipitation. The DNA was then digested with *Bam*HI, at 37 °C for 16 h, phenol/chloroform extracted, ethanol precipitated, electrophoresed in 1% (w/v) agarose gels, and analyzed by Southern blotting, using the *Xba*I/*Bam*HI fragment of pOC3.4 as a probe.

In Situ Hybridization. In situ hybridization analysis of basal and vitamin D enhanced gene expression in ROS 17/2.8 cells was carried out as we have previously described (Pockwinse et al., 1992). In summary, cells were grown on Thermanox coverslips, fixed, hybridized with a ^{35}S-labeled rat osteocalcin gene probe (Lian et al., 1989), and subsequently autoradiographed. RNase A (100 μg/ml) treated ROS 17/2.8 cells were used as a negative control.

RESULTS

Detection of DNase I Hypersensitive Regions in Chromatin of the Osteocalcin Gene. It has been reported that ROS 17/2.8 osteosarcoma cells have retained some of the principal properties exhibited by osteoblasts in vivo, including steroid hormone responsiveness and synthesis of the bone-specific protein osteocalcin (Rodan & Noda, 1991). These characteristics led to the extensive use of this cell line to study the regulatory mechanisms involved in osteocalcin gene expression (Rodan & Noda, 1991). Thus, it has been observed that, although these cells express the osteocalcin gene continually, expression increases when the cell cultures become confluent (Bortell et al., 1993; Owen et al., 1990b; Schepmoes et al., 1991; Pockwinse et al., 1993).

Previous studies carried out by our laboratory (Bortell et al., 1992) have suggested the presence of nucleosomes in the osteocalcin gene promoter. In order to examine further the chromatin structure of the osteocalcin gene, we used the indirect end-labeling method (Wu, 1980) to reveal the presence of DNase I hypersensitive sites. We isolated nuclei from proliferating and confluent ROS 17/2.8 cells and incubated them with increasing concentrations of DNase I, to analyze whether changes in chromatin structure accompany the 5-fold-increased level of transcription previously described in confluent ROS cells (Bortell et al., 1993; Owen et al., 1990b; Schepmoes et al., 1991). In both proliferating and confluent cells, the results revealed DNase I hypersensitivity within the 4.3-kb *Bam*HI restriction fragment that contains the entire osteocalcin gene as well as 5′ and 3′ flanking sequences. Using an *Xba*I/*Bam*HI probe (Figure 1C) from the 3′ end of this osteocalcin gene segment, hypersensitive sites were detected only in the immediate 5′ flanking region (Figure 1A). This nuclease hypersensitivity resided in two major regions, designated hypersensitive site I (HSI) and hypersensitive site II (HSII) (Figure 1C). Both hypersensitive domains were upstream of the coding sequence, spanning the basal (TATA/GRE and OC box) and steroid enhancer (VDRE) transcriptional elements. The intensity of the bands was greater in the samples obtained from confluent cultures (Figure 1A), which parallels the higher level of osteocalcin expression observed in these cells (osteocalcin protein levels measured by radioimmunoassay as a reflection of biosynthesis, 4.0 ± 0.7 ng/10^6 cells in proliferating cultures and 12.0 ± 2.6 ng/10^6 cells in confluent cultures).

To determine whether the presence of DNase I hypersensitive sites in the osteocalcin gene promoter is restricted to cells expressing the osteocalcin gene, nuclei were isolated from H4 cells, a rat hepatoma cell line that does not express osteocalcin, and analyzed under the conditions described above. Figure 2 shows an absence of hypersensitive sites within the DNA fragment including the osteocalcin gene and flanking sequences (Figure 2A,B). As an internal control, we also assessed DNase I hypersensitivity in the vicinity of an H4 histone gene in the hepatoma cells. As expected, DNase I hypersensitive sites were present in the histone H4 gene promoter (Figure 2C), consistent with active transcription of this cell cycle regulated histone gene in exponentially growing cells. These results support a functional relationship between the presence of hypersensitive sites I and II and tissue-specific transcription of the osteocalcin gene in bone cells. In a broad biological context, these results reaffirm that actively transcribed genes usually are marked by the presence of nuclease sensitive domains.

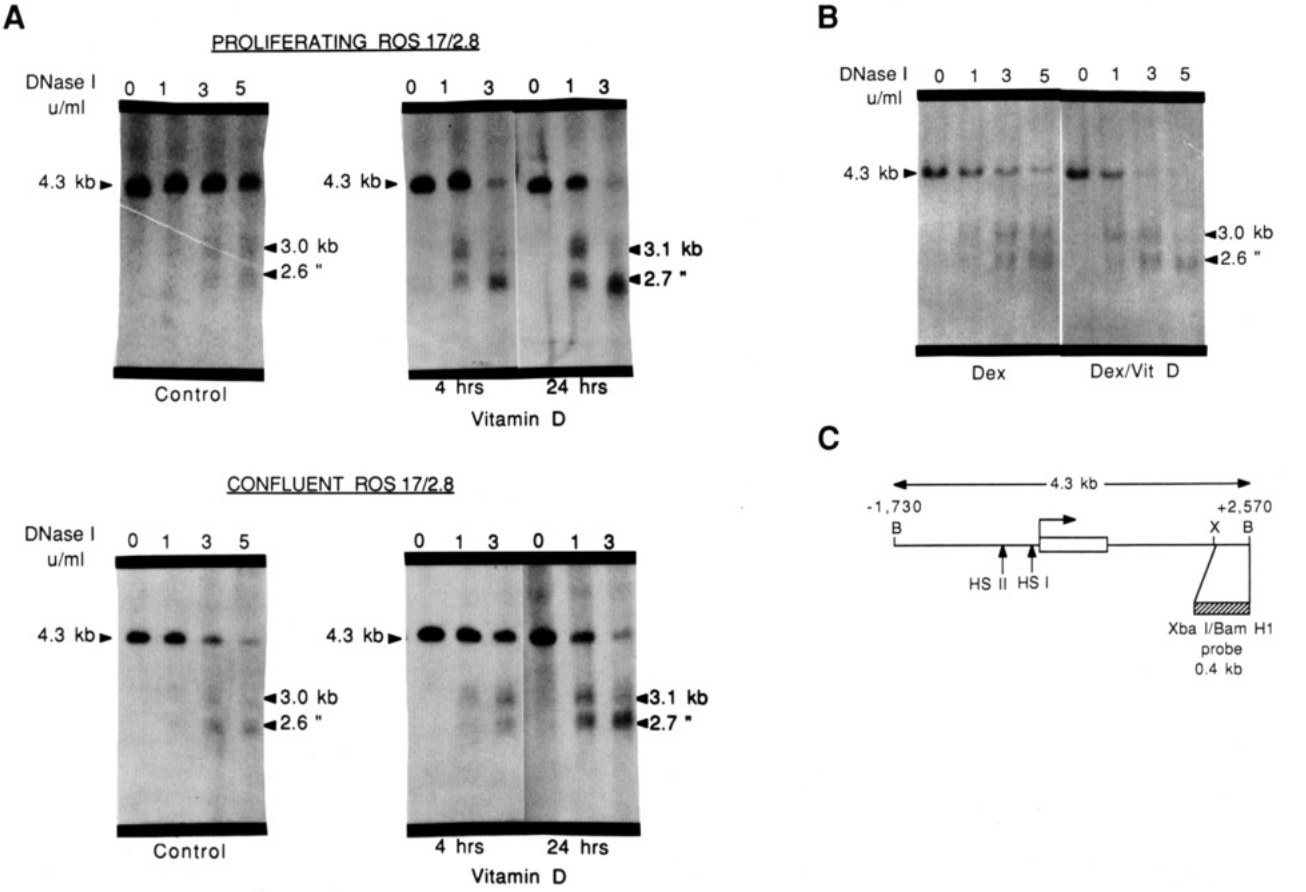

FIGURE 1: DNase I hypersensitivity in the rat osteocalcin gene. Nuclei isolated from rat osteosarcoma cells (ROS 17/2.8), untreated (control) and vitamin D treated for 4 or 24 h (A) or dexamethasone and dexamethasone/vitamin D treated for 24 h (B), were incubated with increasing amounts of DNase I, from 0 to 5 units/ml (indicated over each lane of the blots). The purified DNA was then completely digested with *Bam*HI. Twenty micrograms of each sample was electrophoresed in a 1% agarose gel, blotted, and hybridized with the *Xba*I/*Bam*HI probe (C). Sizes of the nuclease-generated bands are indicated at the right of the blots. DNA size markers are described in Materials and Methods. In the diagram in panel C, the open box represents the osteocalcin gene (including exons and introns), and the horizontal arrow over the gene marks the direction of transcription.

It has been reported that DNase I possesses specificity for AT-rich sequences of DNA (Nelson et al., 1979). Therefore, as an additional control, purified genomic DNA from ROS 17/2.8 cells was partially digested with DNase I and then analyzed by Southern blot using the *Xba*I/*Bam*HI probe illustrated in Figure 1C. As shown in Figure 3, no specific bands were generated by nuclease digestion. This result indicates that the hypersensitivity described above for the osteocalcin gene promoter in intact nuclei of ROS 17/2.8 cells reflects the chromatin structure of the 5′ regulatory domains and is not an inherent property of the DNA sequence.

Effect of Steroid Hormones on DNase I Hypersensitivity of the Osteocalcin Gene. Several laboratories have reported changes in nuclease hypersensitivity of genes following treatment of cells with steroid hormones (Hager et al., 1984; Kaye et al., 1986; Zaret & Yamamoto, 1984). Most of these changes have been observed at or around steroid receptor binding elements and are usually paralleled by induction or repression of the gene. We and other laboratories have described an increased expression of the osteocalcin gene in ROS 17/2.8 cells following vitamin D treatment, at the transcriptional, mRNA, and protein synthesis levels (Demay et al., 1990; Gundberg et al., 1984; Lian et al., 1989; Markose et al., 1990; Owen et al., 1991; Price & Baukol, 1980; Terpening et al., 1991). We have also described an inhibition of this vitamin D induced upregulation of osteocalcin gene expression by treatment of the cells with dexamethasone (Bortell et al., 1993; Schepmoes et al., 1991). On the basis of these findings, we experimentally addressed the effects of vitamin D and dexamethasone on the DNase I hypersensitivity pattern of the osteocalcin gene promoter. We observed that 4 h after vitamin D was added to the culture media both proliferating and confluent cultures of ROS cells responded with a marked increase in hypersensitivity at two promoter domains, as reflected by increased intensity of the bands spanning HSI and HSII (Figure 1A). This increased hypersensitivity remained after 24 h of vitamin D treatment and paralleled the persistence of elevated levels of osteocalcin gene transcription, mRNA, and protein synthesis. Thus, nuclease accessibility of sites in the osteocalcin gene promoter is functionally related to the extent to which the osteocalcin gene is transcribed. No additional hormone responsive hypersensitive sites were detected within the 4.3-kb restriction fragment containing the osteocalcin gene, nor was hypersensitivity induced after vitamin D treatment of cells not expressing osteocalcin (data not shown). Dexamethasone, alone or together with vitamin D (Figure 1B), also did not change the number of DNase I hypersensitive sites found in control or vitamin D treated ROS 17/2.8 cells (Figure 1A). However, the decreased intensity of the 3.0- and 2.6-kb subbands reflected the inhibitory effect of dexamethasone on vitamin D dependent enhancement of osteocalcin gene transcription previously described (Bortell et al., 1993; Schepmoes et al., 1991). The slight reduction in the intensity of the 4.3-kb genomic fragment in Figure 1B compared with Figure 1A represents only an experimental variation due probably to a less efficient hybridization. This

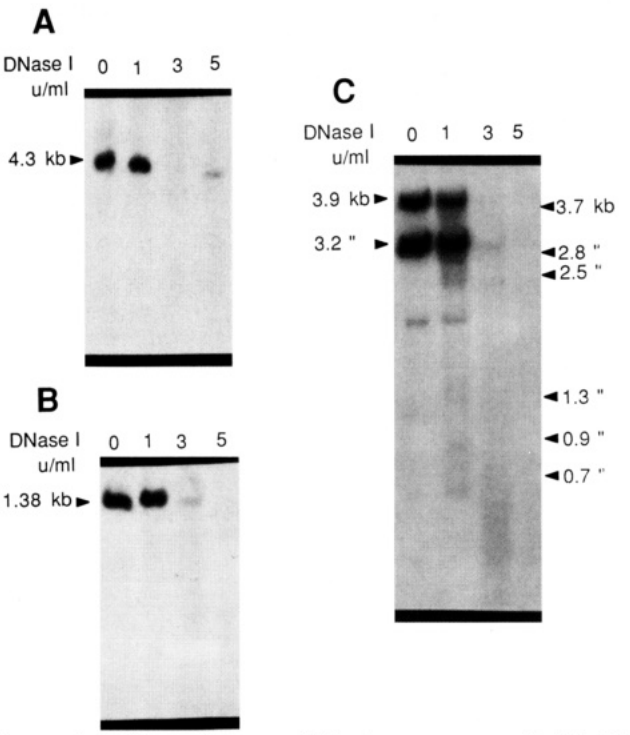

FIGURE 2: DNase I hypersensitivity in nonosseous cells. Nuclei isolated from rat hepatoma cells were incubated with DNase I (concentrations are indicated over each lane), as described in Materials and Methods. (A) Genomic DNA digested with *Bam*HI and hybridized with the *Xba*I/*Bam*HI probe shown in Figure 1C. (B) Genomic DNA digested with *Apa*I and hybridized with the *Ava*I/*Apa*I probe shown in Figure 5B. (C) Genomic DNA digested with *Hin*dIII and hybridized with a *Hin*dIII/*Ava*I probe for a rat somatic histone H4 gene (Wolfe et al., 1989).

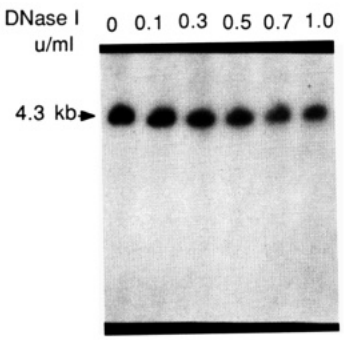

FIGURE 3: Partial DNase I digestion of purified genomic DNA from ROS 17/2.8 cells. DNA (400 μg/ml) was incubated with increasing amounts of DNase I (concentrations are shown above each lane) as described in Materials and Methods. After complete digestion with *Bam*HI, 20 μg of each sample was electrophoresed in a 1% agarose gel, blotted, and hybridized with the *Xba*I/*Bam*HI probe shown in Figure 1C.

does not change the above interpretation since a control blot containing confluent ROS 17/2.8 cell culture samples analyzed simultaneously gave a similar pattern (not shown).

There are several possible interpretations of the increased hypersensitivity in the osteocalcin gene promoter in vitamin D treated ROS 17/2.8 cells. These include an increased level of expression per cell or an increased percentage of expressing cells. To address the relationship between increased hypersensitivity and vitamin D mediated transcriptional upregulation, we examined expression of the osteocalcin gene at the single cell level by in situ hybridization analysis. Although a few cells in untreated cultures had high levels of osteocalcin mRNA, *all cells* in the culture showed some level of osteocalcin mRNA expression. In situ hybridization analyses demonstrated an increased level of expression in all cells throughout the culture in response to vitamin D (Figure 4).

Mapping DNase I Hypersensitive Sites in the Osteocalcin Gene Promoter. Nuclease sensitive regions are segments of DNA that generally span approximately 200 base pairs or more and are composed of several "hot spots" that represent DNase I hypersensitive sites (Elgin, 1988; Gross & Garrard, 1988). To obtain a more accurate mapping of the hypersensitive sites in the osteocalcin gene promoter, we performed digestion of genomic DNA with the restriction enzyme *Apa*I instead of *Bam*HI. Furthermore, hybridization was carried out with an *Ava*I/*Apa*I probe (Figure 5B), instead of the *Xba*I/*Bam*HI probe used in the chromatin structure analyses shown in Figure 1C. The rationale behind this approach was that *Apa*I digestion generates a 1.38-kb genomic fragment, which includes most of the coding sequence and about 0.8 kb of the promoter of the osteocalcin gene (Figure 5B). This strategy permits detection of hypersensitive band sizes in the range of 0.5–1 kb, which can be easily resolved by 2% agarose electrophoresis. Figure 5 shows that the band spanning HSI in Figure 1 can now be resolved into two major subbands of 0.58 and 0.68 kb, which correspond to hypersensitive sites localized at –70 and –170, flanking the OC box (illustrated in Figure 6). These subbands are present in proliferating and confluent ROS 17/2.8 cells, both control and vitamin D treated. Increased intensity of the bands was observed in vitamin D treated cells, similar to the results obtained using *Bam*HI digestion (Figure 1). In contrast, this strategy did not clearly resolve HSII into well-defined subbands. A broad segment of about 130 nucleotides (–390 to –520) in proliferating ROS 17/2.8 cells, or of 200 nucleotides (–390 to –590) in confluent control and in proliferating and confluent vitamin D treated cells, was detected (Figure 5A). This region, which spans the vitamin D responsive element (VDRE), increased in intensity by 4 h after vitamin D treatment.

DISCUSSION

Specific arrangements of chromatin structure have been associated with regulated expression of eukaryotic genes (Elgin, 1988, 1990; Felsenfeld, 1992; Gross & Garrard, 1988). These unique structural features have generally been studied by determining the accessibility of regulatory regions to specific enzymatic activities, namely, DNase I, micrococcal nuclease, and restriction endonucleases (Gross & Garrard, 1988). We have taken advantage of these well-described techniques and studied the accessibility to DNase I in the osteocalcin gene promoter in ROS 17/2.8 cells, which express osteocalcin continually and additionally exhibit upregulation postproliferatively and in response to steroid hormones (Bortell et al., 1993; Owen et al., 1990b, 1993; Schepmoes et al., 1991). Consistent with well-documented nuclease hypersensitivity within promoter domains of several genes that are actively transcribed, our results demonstrate that two DNase I hypersensitive sites (HSI and HSII) are present in the promoter region of the osteocalcin gene. HSI spans basal transcriptional regulatory elements that include the TATA box (–44 to –31) and the OC box (–113 to –76) (Lian et al., 1989; Owen et al., 1990b), and HSII spans the vitamin D responsive element (VDRE, –466 to –437) (Bortell et al., 1993; Demay et al., 1990; Markose et al., 1990; Owen et al., 1990b, 1993; Terpening et al., 1991). The presence and intensity of these two hypersensitive sites are clearly a function of transcriptional activity of the osteocalcin gene, on the basis of observations which include the following: (1) an absence of hypersensitivity both in nonosseous cells and in purified DNA, (2) an increased intensity of the hypersensitive sites with upregulation of

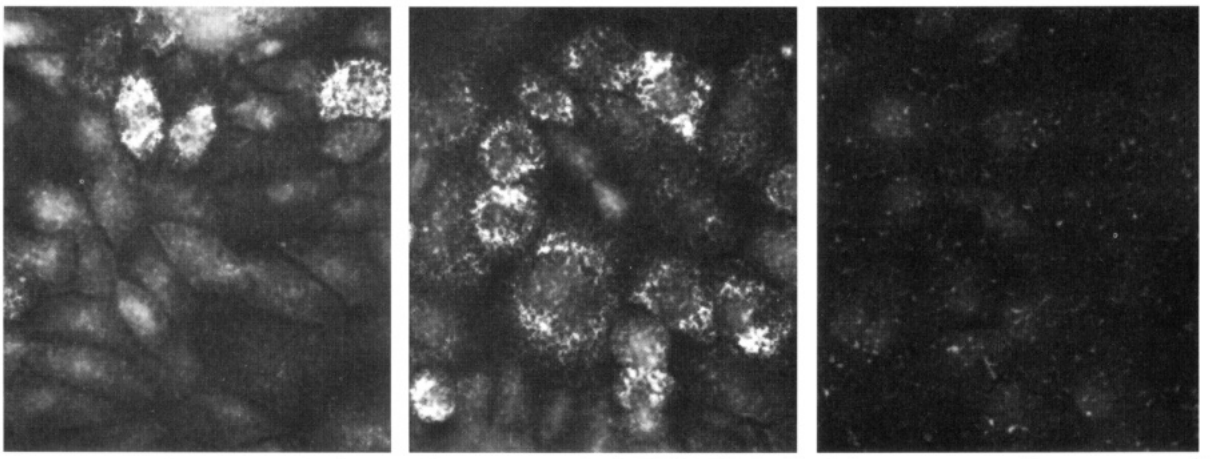

FIGURE 4: In situ hybridization analysis of osteocalcin gene expression in ROS 17/2.8 cells. Cells were grown on Thermanox coverslips and hybridized with a ^{35}S-labeled rat osteocalcin gene probe as described in Materials and Methods. Left panel: Untreated control. Center panel: Vitamin D treated for 24 h. Right panel: Ribonuclease control. Photographs are negative images projected from 35-mm color slides.

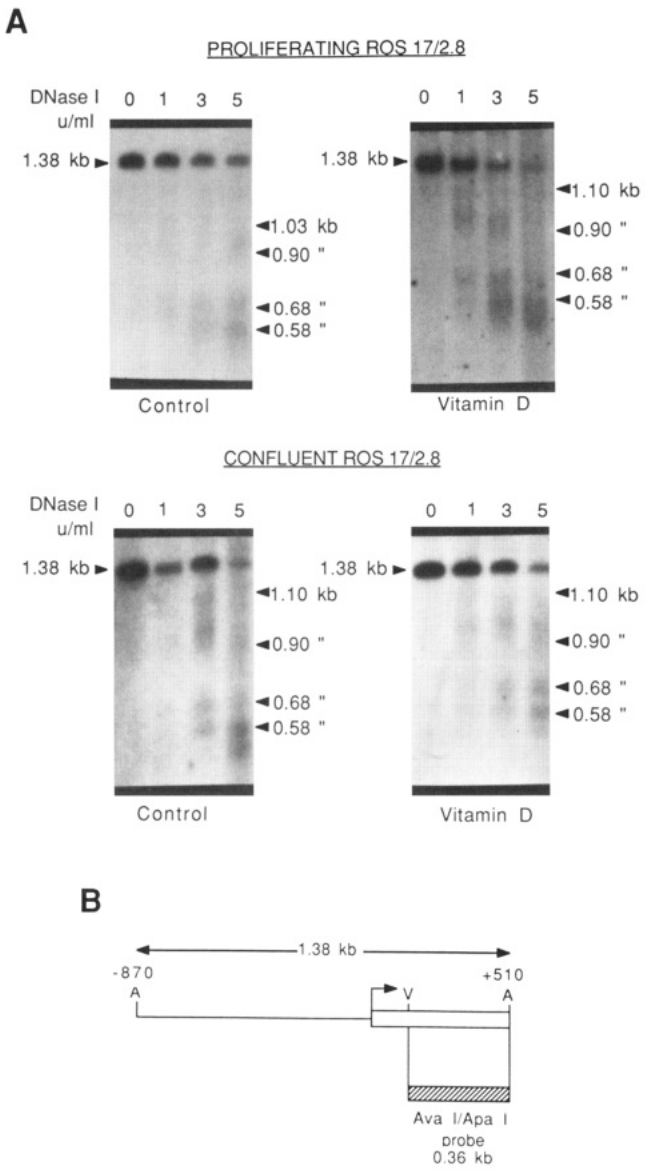

FIGURE 5: Mapping of DNase I hypersensitive sites in the 5′ region of the osteocalcin gene. DNA samples obtained as described in Figure 1 were (A) digested with *Apa*I, electrophoresed in 2% agarose gels, blotted, and hybridized with (B) the *Ava*I/*Apa*I probe. See the caption of Figure 1 for an explanation of the symbols.

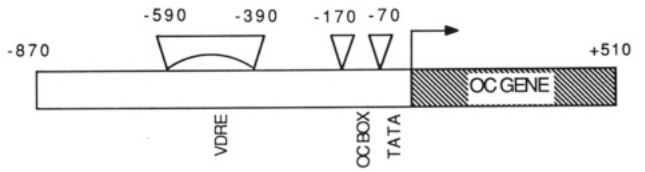

FIGURE 6: Diagram showing the positions of the DNase I hypersensitive sites in the ROS 17/2.8 cell osteocalcin promoter. The vertical arrowheads indicate the positions of the hypersensitive sites, and the horizontal arrow indicates the direction of transcription.

transcription postproliferatively and following vitamin D treatment, and (3) decreased hypersensitivity in dexamethasone-treated cells when osteocalcin transcription is downregulated. Although the degree of hypersensitivity shown here is smaller than that observed in some of the other systems where this method has been applied (Lawson et al., 1980; Anderson et al., 1983; Lu et al., 1992), it appears to be at least similar to that found in most genes so far analyzed (Chrysogelos et al., 1989; Kaye et al., 1986; Mollers et al., 1992). This variation in nuclease hypersensitivity seems then to be a function of cell type and the extent to which a gene is transcribed.

It remains to be established whether the observed relationships of DNase I hypersensitivity in the osteocalcin gene promoter to transcriptional activity are consequences of sequence-specific factor interactions at the OC box and VDRE domains. Equally important is whether structural modifications in chromatin reflected by nuclease accessibility render these basal and steroid hormone enhancer elements competent for promoter factor binding (Hager et al., 1984). Here, consideration must be given to the contribution of nucleotide sequences as well as protein/DNA interactions as potential components of regulatory mechanisms. Unquestionably, the placement of nucleosomes and protein/protein as well as protein/DNA interactions influences the components of chromatin structure revealed by nuclease accessibility. However, there are examples where sequences are at least in part determinants of hypersensitivity. Elgin and co-workers (Lu et al., 1992) have recently demonstrated that pyrimidine-rich segments in the promoter of the *Drosophila melanogaster* heat-inducible hsp 26 gene are determinants of hypersensitivity which appear to be functionally related to transcription. The contribution of sequence-related structural cues, together with sequence-specific binding proteins, may play a role in regulating nuclease accessibility.

The two hypersensitive sites in the osteocalcin gene promoter are located in regulatory domains where a series of protein/DNA interactions have been documented. These interactions

are required for modulation of transcriptional activity in response to a diverse series of physiological mediators of osteocalcin gene expression. Such modulation of gene expression occurs within the context of osteoblast differentiation and maintenance of the bone cell phenotype. For example, integration of regulatory signals at the osteocalcin gene promoter by vitamin D and dexamethasone involves synergistic and/or antagonistic responsiveness at multiple independent promoter regulatory elements. It is reasonable to postulate that structural parameters of the osteocalcin gene promoter in the nuclei of intact cells may facilitate integration of regulatory activities at the proximal basal regulatory domains (TATA/GRE and OC box) and at the VDRE (Bortell et al., 1993). The presence of nucleosomes may reduce the distance between these regulatory elements. Additionally, when the osteocalcin gene is transcriptionally active, structural constraints on the proximal promoter may be imposed by sequence- or tissue-specific interactions with components of the nuclear matrix (Bidwell et al., 1993).

A three-dimensional model for transcriptional regulation of the *Drosophila* hsp 26 gene has also been proposed (Cartright & Elgin, 1986; Elgin, 1988) in which chromatin organization supports the interaction of two spaced, temperature-induced protein–DNA complexes, each involving DNase I hypersensitive sites (+1 to −100 and −300 to −400) (Elgin, 1988). The placement of a nucleosome between the two DNase I hypersensitive sites is proposed to provide a conformation that is competent for interactions of cognate promoter binding factors (Lu et al., 1992, 1993).

Further analysis of nuclease hypersensitivity in relationship to permissiveness for binding of sequence-specific proteins in the osteocalcin promoter will increase our understanding of transcriptional control in relation to chromatin structure, a principal component of three-dimensional nuclear architecture.

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