Journal of Cellular Biochemistry 72:586–594 (1999)

Phosphorylation-Mediated Control of Chromatin Organization and Transcriptional Activity of the Tissue-Specific Osteocalcin Gene

Martin Montecino,[2] **André J. van Wijnen,**[1] **Jane B. Lian,**[1] **Janet L. Stein,**[1] **and Gary S. Stein**[1]*

[1]Department of Cell Biology & Cancer Center, University of Massachusetts Medical Center, Worcester, Massachusetts 01655
[2]Departamento de Biologia Molecular, Facultad de Ciencias Biologicas, Universidad de Concepcion, Concepcion, Chile

Abstract We have analyzed the linkage of protein phosphorylation to the remodeling of chromatin structure that accompanies transcriptional activity of the rat osteocalcin (OC) gene in bone-derived cells. Short incubations with okadaic acid, an inhibitor of protein phosphatases 1 and 2A, induced marked changes in the chromatin organization of the OC gene promoter. These changes were reflected by loss of the two DNase I hypersensitive sites normally present in bone-derived cells expressing this gene. These hypersensitive sites include the elements that control basal tissue-specific expression, as well as steroid hormone regulation. Indeed, the absence of hypersensitivity was accompanied by inhibition of basal and vitamin D-dependent enhancement of OC gene transcription. The effects of okadaic acid on OC chromatin structure and gene activity were specific and reversible. Staurosporine, a protein kinase C inhibitor, did not significantly affect transcriptional activity or DNase I hypersensitivity of the OC gene. We conclude that cellular phosphorylation-dephosphorylation events distinct from protein kinase C-dependent reactions are required for both chromatin remodeling and transcriptional activity of the OC gene in osseous cells. J. Cell. Biochem. 72:586–594, 1999. © 1999 Wiley-Liss, Inc.

Key words: osteocalcin gene; chromatin structure; transcriptional regulation; okadaic acid

The packaging of DNA sequences into nucleosomes and higher-order chromatin structures is a key molecular mechanism responsible for the control of transcriptional activity in eukaryotic cells [Zlatanova and van Holde, 1992]. It is widely accepted that nucleosomes modulate accessibility of specific transcription factors to their cognate promoter binding sequences. Moreover, gene activity is often accompanied by changes in the nucleosomal array, as has been traditionally evidenced by increases in the accessibility to nucleases of specific promoter and enhancer elements [Elgin, 1988; Gross and Garrard, 1988]. Among the mechanisms by which eukaryotic cells can induce alterations in chromatin structure, post-translational modifications have been implicated for several years. Thus, it has been reported that histone acetylation and protein phosphorylation promote marked changes in nucleosomal structure that alter accessibility to specific gene regulatory elements [Bradbury, 1992].

The osteocalcin (OC) gene encodes a 10 kDa bone-specific protein that is induced in osteoblasts at the onset of mineralization at late stages of differentiation [Aronow et al., 1990; Owen et al., 1990]. Transcription of the OC gene is controlled by modularly organized basal regulatory sequences and hormone-responsive enhancer elements [Hoffmann et al., 1994, 1996; Towler et al., 1994a,b; Newberry et al., 1997; Tamura and Noda, 1994; Bidwell et al., 1993; Merriman et al., 1995; Ducy and Karsenty, 1995; Banerjee et al., 1996; Bortell et al., 1992; Demay et al., 1990; Markose et al., 1990; Terpening et al., 1991; Guo et al., 1995; Breen et al., 1994]. These elements are located within two

Contract grant sponsor: FONDECYT; Contract grant number: 1971077; Contract grant sponsor: DIUC; Contract grant number: 96031.071–1; Contract grant sponsor: National Institutes of Health; Contract grant number: AR45698.

*Correspondence to the authors at: Department of Cell Biology & Cancer Center, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, MA 01655.

Received 18 September 1998; Accepted 29 October 1998

© 1999 Wiley-Liss, Inc.

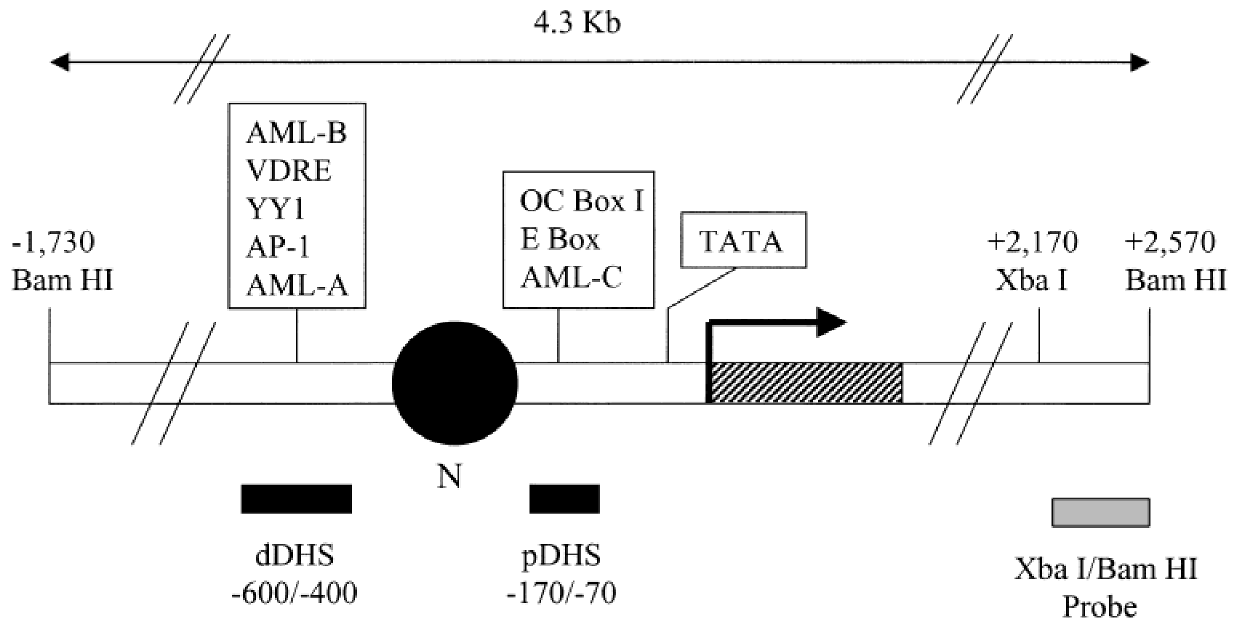


Fig. 1. Schematic representation of the rat OC gene and flanking regions. The diagram shows the Xba I/Bam HI DNA segment used as hybridization probe in the indirect end-labeling analysis. The hatched box represents the OC gene coding region (including exons and introns) and the horizontal arrow above the gene marks the direction of transcription. The filled circle represents a positioned nucleosome (N) and the filled boxes below, the distal (dDHS) and proximal (pDHS) DNase I hypersensitive sites. The locations of key transcription regulatory elements within both DNase I hypersensitive sites are also marked.

DNase I hypersensitive sites (Fig. 1) that are present only in bone-derived cells expressing this gene [Montecino et al., 1994, 1996]. Thus, remodeling of the chromatin structure of the OC gene promoter accompanies transcriptional activation during osteoblast differentiation [Montecino et al., 1996]. However, regulatory mechanisms that mediate these conformational modifications in nucleosomal organization of the OC gene promoter have not been experimentally addressed. We have recently reported that hyperacetylation of nuclear proteins alters the chromatin organization of the OC gene promoter and prevents vitamin D-mediated transcriptional upregulation [Montecino et al., in press]. By combining nuclease accessibility, indirect end-labeling, and ligation-mediated PCR analysis, we determined that protein-DNA and protein-protein interactions that promote formation of the distal DNase I hypersensitive site do not occur under conditions of hyperacetylation.

It was previously shown that perturbing phosphorylation pathways blocked vitamin D enhancement of transiently transfected OC promoter-CAT constructs in ROS 17/2.8 cells [Desai et al., 1995]. Thus, treatment of these bone-derived cells that continuously express OC, with okadaic acid, a protein phosphatase inhibitor, resulted in a marked decrease in binding of the vitamin D receptor complex (VDR-RXR) to the OC gene vitamin D responsive element (VDRE). In contrast, gel mobility shift assays using nuclear extracts from ROS 17/2.8 cells treated with staurosporine demonstrated that inhibition of protein kinase C-mediated phosphorylation pathways did not impair competency of the VDR-RXR complex to interact with the VDRE. Moreover, it was reported that treatment with either okadaic acid or staurosporine induced only subtle effects on the binding of AP-1, Sp1, and ATF to their respective DNA recognition elements [Desai et al., 1995].

We have now determined that alterations in phosphorylation events induced in ROS 17/2.8 cells by okadaic acid affect the chromatin organization and transcriptional activity of the endogenous OC gene. We report that treatment with okadaic acid had a marked effect on the DNase I hypersensitivity normally observed in the OC gene promoter in bone-derived cells [Montecino et al., 1996]. A significant decrease in hypersensitivity at both distal (−600 to −400) and proximal (−170 to −70) sites occurred following 4 h of incubation. This effect was found to be reversible and accompanied by a marked

decrease in basal OC transcriptional activity as measured by nuclear run on assays.

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 reported [Majeska et al., 1980]. Confluent cultures were treated with 100 nM okadaic acid, 100 nM staurosporine, 10^{-8} M, 1,25- dihydroxyvitamin D3, or combinations of them, for various periods of time as indicated. OC gene transcription was determined by nuclear run on analysis [Montecino et al., 1996]. OC biosynthesis was assessed by measuring the OC secreted into the medium by RIA, as previously described [Gundberg et al., 1984].

DNase I Hypersensitivity Analysis

DNase I digestion analysis of isolated nuclei from confluent cultures of ROS 17/2.8 cells was performed according to the indirect end-labeling method [Wu, 1980]. Restriction enzyme cleavage of purified genomic DNA, electrophoresis, blotting, and hybridization were performed as previously described [Montecino et al., 1994, 1996]. As shown in Figure 1, we used the Xba I/Bam HI probe from the 3' end of the plasmid pOC3.4 to analyze DNase I hypersensitivity of the OC gene. To determine the hypersensitivity pattern associated with the histone H4 genes, we used a probe directed against the coding region of the rat histone H4t gene (a generous gift from Sidney Grimes, Louisiana State University).

RESULTS

Okadaic Acid Treatment Induces a Decrease in Hypersensitivity of the Osteocalcin Gene Promoter

Extensive studies have shown that in eukaryotic cells most active genes exhibit DNase I hypersensitive sites, usually within the promoter region. These domains normally reflect alterations in the canonical nucleosomal organization and the interaction of nuclear factors with their specific DNA recognition elements [Elgin, 1988; Gross and Garrard, 1988]. We have shown that formation of two DNase I hypersensitive sites accompanies transcriptional activation of the OC gene in bone-derived cells [Montecino et al., 1994, 1996]. The formation and intensity of these sites reflect transcriptional activity of the OC gene since: 1) they were not detected in non-osseous cells or in bone-derived cells which do not express this gene, and 2) they are increased following vitamin D treatment, which results in a significant enhancement of basal OC gene transcription.

It has been reported that altering phosphorylation pathways in ROS 17/2.8 cells, by inhibiting either protein kinase or phosphatase activities, results in significantly decreased levels of expression from transiently transfected OC promoter constructs [Desai et al., 1995]. In order to assess the role of protein phosphorylation-dephosphorylation events in the remodeling of the chromatin organization of the endogenous OC gene promoter, we measured the effect of incubating ROS 17/2.8 cells with okadaic acid (an inhibitor of protein phosphatases 1 and 2A) or staurosporine (a protein kinase C inhibitor) on the formation of the DNase I hypersensitive sites. As shown in Figure 2, treatment of confluent ROS 17/2.8 cell cultures with 100 nM okadaic acid for 4 h resulted in a significant decrease in DNase I hypersensitivity at the OC gene promoter. This effect was less significant in cells co-treated with 10^{-8} M vitamin D3 (Fig. 2A). When the treatment with okadaic acid was extended to 24 h (Fig. 2B), both distal and proximal DNase I hypersensitive sites were undetectable, even in the presence of vitamin D3. When protein kinase C activity was inhibited with staurosporine, we observed that neither 4 nor 24 h incubation periods were sufficient to change significantly the chromatin organization of the OC gene promoter.

To analyze if the okadaic acid-induced changes in the OC gene promoter were reversible, we reincubated the 4 h-treated confluent ROS 17/2.8 cultures in fresh okadaic acid-free media. As shown in Figure 2C, within 24 h after removal of okadaic acid, the alterations in the OC gene chromatin structure induced by this treatment were completely reversed and restored to the control condition.

To determine if okadaic acid promoted a generalized change in the chromatin structure of ROS cells, we analyzed the DNase I hypersensitivity pattern associated with the histone H4 genes. Indirect end-labeling analysis using a probe that recognizes the coding region of the rat histone H4 genes [Montecino et al., 1996] demonstrated that incubation of ROS 17/2.8 cells with either okadaic acid or staurosporine

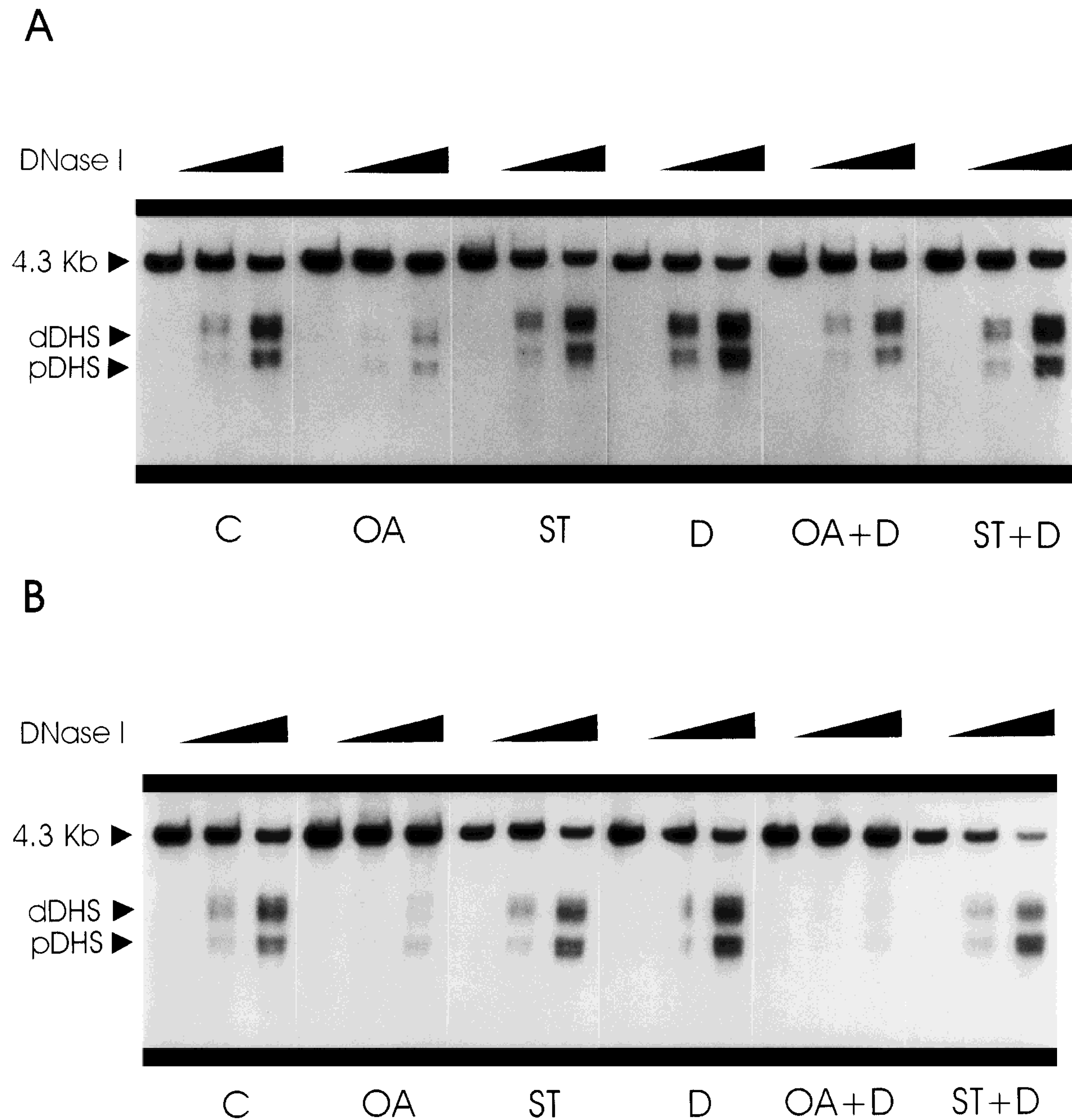
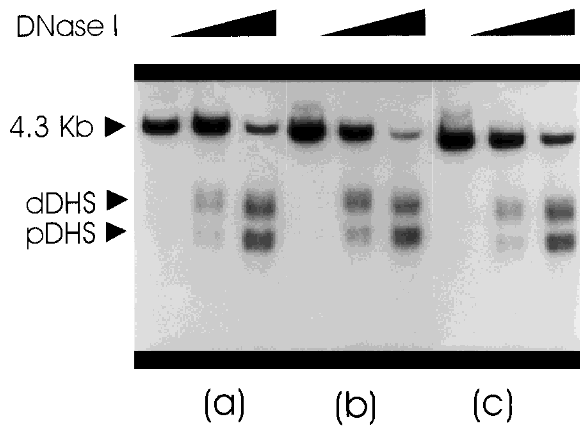


Fig. 2. Okadaic acid treatment inhibits formation of DNase I hypersensitive sites at the OC gene promoter. Confluent ROS 17/2.8 cell cultures were incubated in the presence of 100 nM okadaic acid (OA), 100 nM staurosporine (ST), 10^{-8} M vitamin D3 (D), vitamin D3 and okadaic acid (OA+D), or vitamin D3 and staurosporine (ST+D) combined during 4 **(A)** or 24 **(B)** h. Nuclei were subsequently isolated and incubated with increasing concentrations of DNase I for 10 min. The purified genomic DNA was then completely cleaved with Bam HI, electrophoresed in a 1.2% agarose gel, blotted, and hybridized with the Xba I/Bam HI probe (see Fig. 1). **C:** Confluent ROS 17/2.8 cell cultures were treated for 24 h under control conditions (**a**), or in the presence of 100 nM okadaic acid alone (**b**), or with 10^{-8} M vitamin D3 and okadaic acid combined (**c**). Subsequently, the cultures were washed with fresh media and incubated under control conditions for 20 h. Following nuclei isolation, the DNase I hypersensitivity analysis at the OC gene promoter was carried out by the indirect end-labeling method as described above. **D:** Aliquots of the purified genomic DNA from the samples analyzed in B were completely cleaved with Kpn I and Eco RI, electrophoresed in a 1.5% agarose gel, blotted, and hybridized with a probe that recognizes the coding sequence of rat histone H4 genes [Montecino et al., 1996].



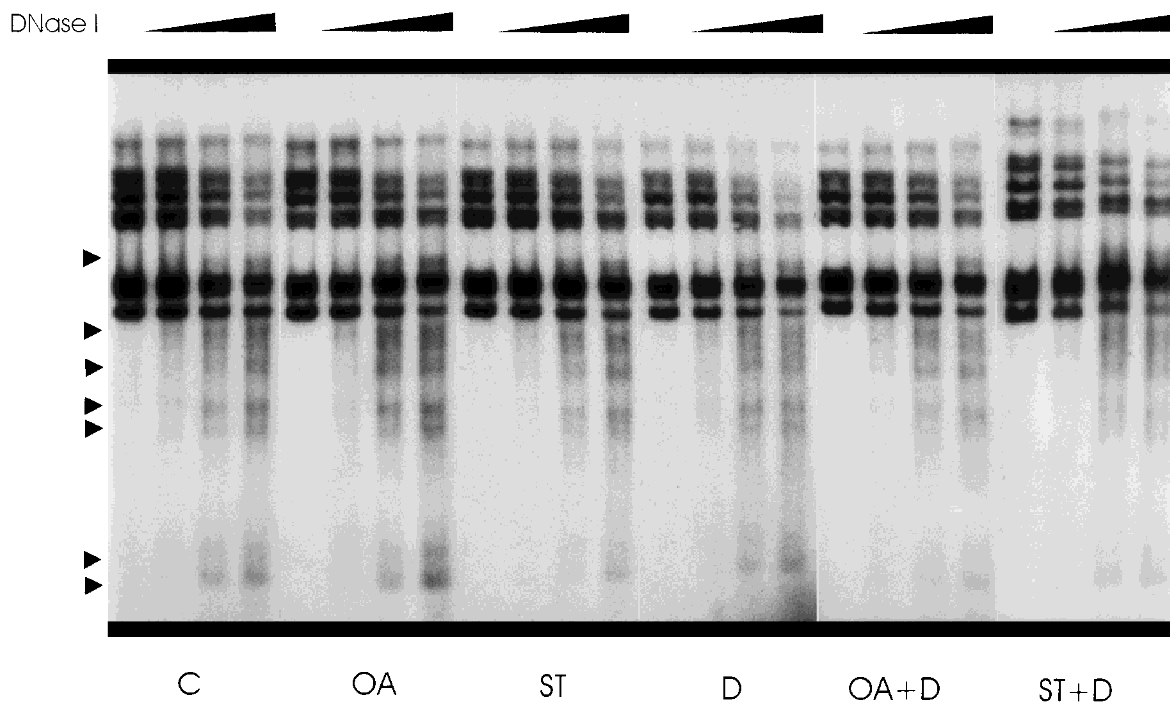


Figure 2. (Continued)

did not significantly alter the hypersensitivity pattern of these genes (Fig. 2D).

Taken together, these results indicate that formation of DNase I hypersensitive sites at the OC gene promoter requires phosphorylation-dephosphorylation reactions. These reactions involve protein phosphatase 1 and 2A activities, but not staurosporine-inhibited protein kinase C activity.

Okadaic Acid Treatment Inhibits Expression of the OC Gene in ROS 17/2.8 Cells

We next evaluated the effect of inhibiting phosphorylation-dephosphorylation events with

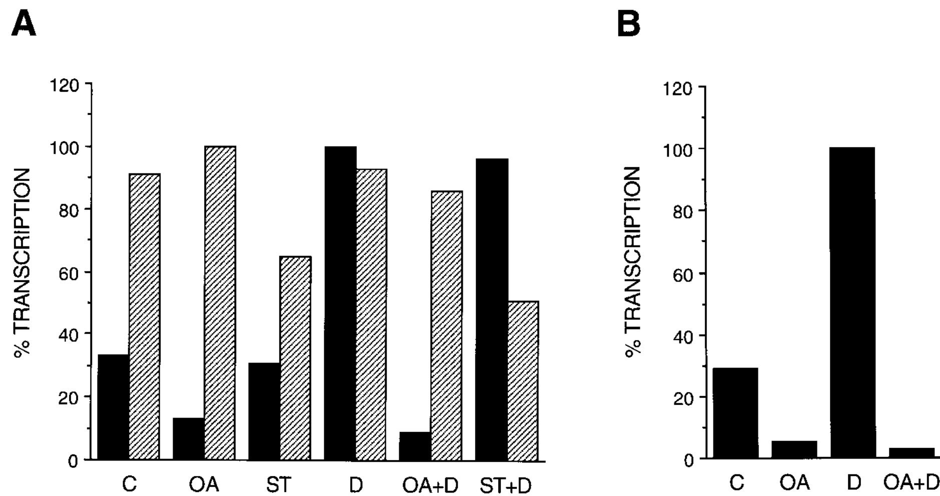


Fig. 3. Okadaic acid treatment inhibits OC gene transcription. Transcriptional activities of the rat OC (solid bars) and histone H4 (hatched bars) genes in ROS 17/2.8 cells cultured under control conditions, and in the presence of okadaic acid or staurosporine alone or in combination with vitamin D3, were measured by nuclear run on analysis. **A:** Effect of 4 h incubation, and (**B**) effect of 24 h incubation. C, control; OA, okadaic acid (100 nM); ST, staurosporine (100 nM); D, vitamin D3 (10^{-8} M). The data presented are representative of results from three independent experiments.

TABLE I. Effect of Okadaic Acid Treatment on the Osteocalcin Biosynthesis Rate in ROS 17/2.8 Cells

Treatment	OC concentration (ng/dl)
Control	35.68 ± 11.34
OA[a]	15.86 ± 11.70
Vit D3[b]	125.29 ± 28.65
OA + Vit D3	45.71 ± 25.56

[a]OA, okadaic acid (100 nM).
[b]Vit D3, Vitamin D3 (10^{-8} M).

okadaic acid and staurosporine on the level of OC gene expression in ROS 17/2.8 cells. For this purpose, we measured transcriptional activity by nuclear run on analysis [Montecino et al., 1996] and OC biosynthesis by ELISA [Gundberg et al., 1984]. We observed that after 4 h of incubation with okadaic acid, there was a marked reduction in the basal levels of OC gene transcription (Fig. 3A). Furthermore, in these cells vitamin D-dependent upregulation of OC gene transcription was completely inhibited. In contrast, transcription of the histone H4 genes was not significantly affected (Fig. 3A). If treatment with okadaic acid was further extended to 24 h, a similar inhibition of OC expression, as reflected by the levels of OC transcription (Fig. 3B) and OC biosynthesis (Table I), was observed. Treatment of confluent ROS 17/2.8 cell cultures with staurosporine did not alter the basal or vitamin D-enhanced transcriptional activity of the OC gene (Fig. 3A).

Taken together, these results indicate that, in agreement with a previous report, but now analyzed within an endogenous chromatin context, inhibition of phosphatases 1 and 2A with okadaic acid prevents basal and vitamin D-enhanced OC transcription in ROS 17/2.8 cells. This inhibition is effective following 4 h of incubation and correlates with marked changes in chromatin organization.

DISCUSSION

We have studied the role of protein phosphorylation-dephosphorylation events in the chromatin remodeling process that accompanies transcriptional activation of the rat OC gene in bone-derived cells. We have measured the effect of incubating confluent ROS 17/2.8 cell cultures with okadaic acid, an inhibitor of protein phosphatases 1 and 2A, and staurosporine, a nonspecific protein kinase C inhibitor [Meggio et al., 1995; Wilkinson et al., 1993]. The effect on chromatin structure was evaluated by analyzing changes in the DNase I hypersensitivity pattern of the OC gene promoter by the indirect end-labeling method. The effect on OC transcriptional activity was measured by nuclear run on assays. We found that short treatments with okadaic acid (4 h) significantly decreased formation of the two DNase I hypersensitive sites normally present at the OC gene promoter

of these cells. In addition, these incubations inhibited basal and vitamin D-dependent enhancement of OC transcription. Our results support the previously reported tight and direct relationship between OC transcriptional activity and the presence of DNase I hypersensitivity in the OC gene promoter region [Montecino et al., 1994, 1996].

It has been well-established that short incubation of cell cultures with okadaic acid results in phosphorylated chromosomal proteins [Guo et al., 1995; Feng et al., 1991; Herrera et al., 1996; Chadee et al., 1997; Mahadevan et al., 1991; Ajiro et al., 1996]. However, the overall effect of this treatment on general chromatin structure remains to be established. Contradictory results have been obtained by different groups. Thus, it was reported that okadaic acid treatment led to chromosome condensation [Guo et al., 1995; Ajiro et al., 1996], reflecting a molecular mechanism that is operative during normal mitosis. These results also suggested that protein phosphatases 1 and 2A may be important in regulating chromosomal condensation by restricting the level of histone phosphorylation during interphase. Premature chromosome condensation may thereby be promoted by inhibiting activity of these phosphatases [Guo et al., 1995]. In contrast, it has also been reported that a histone phosphorylated state induced by incubating cells with okadaic acid leads to a decondensed chromatin structure [Feng et al., 1991; Herrera et al., 1996; Chadee et al., 1997]. It was proposed with this decondensed structure may be required to facilitate transcription and replication processes.

Although a definitive explanation of these apparently contradictory results is not known, it may be related to differences among cell types, especially with respect to sensitivity to okadaic acid. In our studies, we observed that lower concentrations of okadaic acid (50 nM) did not significantly affect the basal or vitamin D-enhanced expression of OC promoter constructs transiently transfected into osteoblastic cells (unpublished results). We also found that treatment with 100 nM okadaic acid for up to 24 h did not change the morphology of these cell cultures and did not significantly increase cell death (unpublished results). In addition, it was previously observed that transcription factor binding activities of AP-1, Sp1, and ATF were not affected following 24 h of incubation with okadaic acid [Desai et al., 1995]. We have also found that treatment with either okadaic acid or staurosporine for 4 h does not alter the vitamin D-dependent DNA-binding activity of the VDR-RXR complex (data not shown). Here we have determined that the changes in the DNase I hypersensitivity pattern of the OC gene promoter are reversible. These changes do not reflect major and generalized alteration of chromatin structure in okadaic acid treated-ROS 17/2.8 cells.

Taken together, these observations suggest that cellular protein phosphorylation-dephosphorylation events have an important role in the molecular mechanism responsible for transcriptional activation and chromatin remodeling at the OC gene promoter in osteoblastic cells. Staurosporine-sensitive protein kinase activities such as protein kinase C do not appear to participate. Interestingly, Haussler and colleagues have previously suggested a role for casein kinase II in the phosphorylation events that precede ligand-dependent binding of the vitamin D3 receptor in vivo [Jurutka et al., 1993, 1996].

It has been recently reported that the catalytic subunit of the Ku-DNA-dependent serine-threonine protein kinase (Ku-DNA PK) complex phosphorylates in vivo hGCN5, a putative transcriptional adapter that possesses histone acetyltransferase (HAT) activity [Barlev et al., 1998]. This phosphorylation inhibited the GCN5 HAT activity. The inhibitory effect was abolished by dephosphorylating GCN5 with a phosphatase. Because the Ku-DNA PK activity has been implicated in transcriptional repression [Giffin et al., 1996; Kuhn et al., 1995], a molecular mechanism for regulating in vivo the role of GCN5 in transcription was suggested [Barlev et al., 1998]. Interestingly, HAT activity has also been directly implicated in the chromatin remodeling events normally associated with gene transcription [Wolffe and Pruss, 1996; Struhl, 1998]. We have recently reported that nonregulated chromatin acetylation has profound effects on both transcriptional activity and DNase I hypersensitivity of the OC gene in ROS 17/2.8 cells [Montecino et al., in press]. We propose that inhibition of protein dephosphorylation events could also alter histone acetylation activities, such as that present in GCN5, which are required for both transcriptional activity and chromatin remodeling of the OC gene in bone-derived cells. The nature of the kinase pathway and the specific kinase activities in-

volved in DNase I hypersensitivity and OC gene expression are currently being investigated.

ACKNOWLEDGMENTS

The authors thank Dr. Rajesh Desai for stimulating discussions, and Rosa Mastrototaro, Jack Green, and Elizabeth Buffone for expert technical assistance. This work was supported by a grant from the National Institutes of Health (NIH). The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. M.M. was also supported by grants from FONDECYT, DIUC, and Fundación Andes, Chile.

REFERENCES

Ajiro K, Yoda K, Utsumi K, Nishikawa Y. 1996. Alteration of cell cycle-dependent histone phosphorylations by okadaic acid. Induction of mitosis-specific H3 phosphorylation and chromatin condensation in mammalian interphase cells. J Biol Chem 271:13197–13201.

Aronow MA, Gerstenfeld LC, Owen TA, Tassinari MS, Stein GS, Lian JB. 1990. Factors that promote progressive development of the osteoblast phenotype in cultured fetal rat calvaria cells. J Cell Physiol 143:213–221.

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 USA 93:4968–4973.

Barlev NA, Poltoratsky V, Owen-Hughes T, Ying C, Liu L, Workman JL, Berger SL. 1998. Repression of GCN5 histone acetyltransferase activity via bromodomain- mediated binding and phosphorylation by the Ku-DNA-dependent protein kinase complex. Mol Cell Biol 18:1349–1358.

Bidwell JP, van Wijnen AJ, Fey EG, Dworetzky S, Penman S, Stein JL, Lian JB, Stein GS. 1993. Osteocalcin gene promoter-binding factors are tissue-specific nuclear matrix components. Proc Natl Acad Sci USA 90:3162–3166.

Bortell R, Owen TA, Bidwell JP, Gavazzo P, Breen E, van Wijnen AJ, DeLuca HF, Stein JL, Lian JB, Stein GS. 1992. Vitamin D-responsive protein-DNA interactions at multiple promoter regulatory elements that contribute to the level of rat osteocalcin gene expression. Proc Natl Acad Sci USA 89:6119–6123.

Bradbury EM. 1992. Reversible histone modifications and the chromosome cell cycle. BioEssays 14:9–16.

Breen EC, van Wijnen AJ, Lian JB, Stein GS, Stein JL. 1994. In vivo occupancy of the vitamin D responsive element in the osteocalcin gene supports vitamin D-dependent transcriptional upregulation in intact cells. Proc Natl Acad Sci USA 91:12902–12906.

Chadee DN, Allis CD, Wright JA, Davie JR. 1997. Histone H1b phosphorylation is dependent upon ongoing transcription and replication in normal and ras-transformed mouse fibroblasts. J Biol Chem 272:8113–8116.

Demay MB, Gerardi JM, DeLuca HF, Kronenberg HM. 1990. DNA sequences in the rat osteocalcin gene that bind the 1,25-dihydroxyvitamin D3 receptor and confer responsive to 1,25-dihydroxyvitamin D3. Proc Natl Acad Sci USA 87:369–373.

Desai RK, van Wijnen AJ, Stein JL, Stein GS, Lian JB. 1995. Control of 1,25-dihydroxyvitamin D3 receptor-mediated enhancement of osteocalcin gene transcription: Effects of perturbing phosphorylation pathways by okadaic acid and staurosporine. Endocrinology 136:5685–5693.

Ducy P, Karsenty G. 1995. Two distinct osteoblast-specific cis-acting elements control expression of a mouse osteocalcin gene. Mol Cell Biol 15:1858–1869.

Elgin SC. 1988. The formation and function of DNase I hypersensitive sites in the process of gene activation. J Biol Chem 263:19259–19262.

Feng JL, Irving J, Villeponteau B. 1991. A phosphatase inhibitor enhances the DNase I sensitivity of active chromatin. Biochemistry 30:4747–4752.

Giffin W, Torrance H, Rodda DJ, Prefontaine GG, Pope L, Hache RJ. 1996. Sequence-specific DNA binding by Ku autoantigen and its effects on transcription. Nature 380: 265–268.

Gross DS, Garrard WT. 1988. Nuclease hypersensitive sites in chromatin. Ann Rev Biochem 57:159–197.

Gundberg CM, Hauschka PV, Lian JB, Gallop PM. 1984. Osteocalcin: Isolation, characterization, and detection. Methods Enzymol 107:516–544.

Guo B, Odgren PR, van Wijnen AJ, Last TJ, Nickerson J, Penman S, Lian JB, Stein JL, Stein GS. 1995. The nuclear matrix protein NMP-1 is the transcription factor YY1. Proc Natl Acad Sci USA 92:10526–10530.

Guo XW, Th'ng JP, Swank RA, Anderson HJ, Tudan C, Bradbury EM, Roberge M. 1995. Chromosome condensation induced by fostriecin does not require p34cdc2 kinase activity and histone H1 hyperphosphorylation, but is associated with enhanced histone H2A and H3 phosphorylation. EMBO J 14:976–985.

Herrera RE, Chen F, Weinberg RA. 1996. Increased histone H1 phosphorylation and relaxed chromatin structure in Rb-deficient fibroblasts. Proc Natl Acad Sci USA 93: 11510–11515.

Hoffmann HM, Catron KM, van Wijnen AJ, McCabe LR, Lian JB, Stein GS, Stein JL. 1994. Transcriptional control of the tissue-specific, developmentally regulated osteocalcin gene requires a binding motif for the Msx family of homeodomain proteins. Proc Natl Acad Sci USA 91:12887–12891.

Hoffmann HM, Beumer TL, Rahman S, McCabe LR, Banerjee C, Aslam F, Tiro JA, van Wijnen AJ, Stein JL, Stein GS, Lian JB. 1996. Bone tissue-specific transcription of the osteocalcin gene: Role of an activator osteoblast-specific complex and suppressor hox proteins that bind the OC box. J Cell Biochem 61:310–324.

Jurutka PW, Terpening CM, Haussler MR. 1993. The 1,25-dihydroxy-vitamin D3 receptor is phosphorylated in response to 1,25-dihydroxy-vitamin D3 and 22-oxacalcitriol in rat osteoblasts, and by casein kinase II, in vitro. Biochemistry 32:8184–8192.

Jurutka PW, Hsieh JC, Nakajima S, Haussler CA, Whitfield GK, Haussler MR. 1996. Human vitamin D receptor phosphorylation by casein kinase II at Ser-208 potentiates transcriptional activation. Proc Natl Acad Sci USA 93:3519–3524.

Kuhn A, Gottlieb TM, Jackson SP, Grummt I. 1995. DNA-dependent protein kinase: A potent inhibitor of transcription by RNA polymerase I. Genes Dev 9:193–203.

Mahadevan LC, Willis AC, Barratt MJ. 1991. Rapid histone H3 phosphorylation in response to growth factors, phorbol esters, okadaic acid, and protein synthesis inhibitors. Cell 65:775–783.

Majeska RJ, Rodan SB, Rodan GA. 1980. Parathyroid hormone-responsive clonal cell lines from rat osteosarcoma. Endocrinology 107:1494–1503.

Markose ER, Stein JL, Stein GS, Lian JB. 1990. Vitamin D-mediated modifications in protein-DNA interactions at two promoter elements of the osteocalcin gene. Proc Natl Acad Sci USA 87:1701–1705.

Meggio F, Donella Deana A, Ruzzene M, Brunati AM, Cesaro L, Guerra B, Meyer T, Mett H, Fabbro D, Furet P. 1995. Different susceptibility of protein kinases to staurosporine inhibition. Kinetic studies and molecular bases for the resistance of protein kinase CK2. Eur J Biochem 234:317–322.

Merriman HL, van Wijnen AJ, Hiebert S, Bidwell JP, Fey E, Lian J, Stein J, Stein GS. 1995. The tissue-specific nuclear matrix protein, NMP-2, is a member of the AML/CBF/PEBP2/runt domain transcription factor family: Interactions with the osteocalcin gene promoter. Biochemistry 34:13125–13132.

Montecino M, Pockwinse S, Lian J, Stein G, Stein J. 1994. DNase I hypersensitive sites in promoter elements associated with basal and vitamin D dependent transcription of the bone-specific osteocalcin gene. Biochemistry 33:348–353.

Montecino M, Lian J, Stein G, Stein J. 1996. Changes in chromatin structure support constitutive and develomentally regulated transcription of the bone-specific osteocalcin gene in osteoblastic cells. Biochemistry 35:5093–5102.

Montecino M, Frenkel B, van Wijnen A, Lian J, Stein G, Stein J. Chromatin hyperacetylation abrogates vitamin D-mediated transcriptional upregulation of the tissue-specific osteocalcin gene in vivo. Biochemistry, in press.

Newberry EP, Boudreaux JM, Towler DA. 1997. Stimulus-selective inhibition of rat osteocalcin promoter induction and protein-DNA interactions by the homeodomain repressor Msx2. J Biol Chem 272:29607–29613.

Owen TA, Aronow M, Shalhoub V, Barone LM, Wilming L, Tassinari MS, Kennedy MB, Pockwinse S, Lian JB, Stein GS. 1990. Progressive development of the rat osteoblast phenotype in vitro: Reciprocal relationships in expression of genes associated with osteoblast proliferation and differentiation during formation of the bone extracellular matrix. J Cell Physiol 143:420–430.

Struhl K. 1998. Histone acetylation and transcriptional regulatory mechanisms. Genes Dev 12:599–606.

Tamura M, Noda M. 1994. Identification of a DNA sequence involved in osteoblast-specific gene expression via interaction with helix-loop-helix (HLH)-type transcription factors. J Cell Biol 126:773–782.

Terpening CM, Haussler CA, Jurutka PW, Galligan MA, Komm BS, Haussler MR. 1991. The vitamin D-responsive element in the rat bone Gla protein gene is an imperfect direct repeat that cooperates with other cis-elements in 1,25-dihydroxyvitamin D3-mediated transcriptional activation. Mol Endocrinol 5:373–385.

Towler DA, Bennett CD, Rodan GA. 1994a. Activity of the rat osteocalcin basal promoter in osteoblastic cells is dependent upon homeodomain and CP1 binding motifs. Mol Endocrinol 8:614–624.

Towler DA, Rutledge SJ, Rodan GA. 1994b. Msx-2/Hox 8.1: A transcriptional regulator of the rat osteocalcin promoter. Mol Endocrinol 8:1484–1493.

Wilkinson SE, Parker PJ, Nixon JS. 1993. Isoenzyme specificity of bisindolylmaleimides, selective inhibitors of protein kinase C. Biochem J 294:335–337.

Wolffe AP, Pruss D. 1996. Targeting chromatin disruption: transcription regulators that acetylate histones. Cell 84: 817–819.

Wu C. 1980. The 5' ends of Drosophila heat shock genes in chromatin are hypersensitive to DNase I. Nature 286: 854–860.

Zlatanova JS, van Holde KE. 1992. Chromatin loops and transcriptional regulation. Crit Rev Eukaryot Gene Exp 2:211–224.