Transcriptional Mechanisms of Bone Morphogenetic Protein-induced Osteoprotegrin Gene Expression*

Mei Wan, Xingming Shi, Xu Feng, and Xu Cao‡

From the Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama 35294

Osteoprotegerin (OPG), an osteoblast-secreted decoy receptor, specifically binds to osteoclast differentiation factor and inhibits osteoclast maturation. Members of the transforming growth factor-β superfamily including bone morphogenetic proteins (BMPs) stimulate OPG mRNA expression. In this study, we have characterized the transcriptional mechanisms of BMP-induced OPG gene expression. Transfection of Smad1 and a constitutively active BMP type IA receptor ALK3 (Q233) stimulated the OPG promoter. Deletion analysis of the OPG promoter identified two Hoxc-8 binding sites that respond to BMP stimulation. Glutathione S-transferase-Hoxc-8 protein binds to these two Hox sites specifically. Consistent with the transfection results of the native promoter, ALK3 or Smad1 linker region, which interacts with Hoxc-8, stimulated the activation of the reporter construct with the two Hox sites. Overexpression of Hoxc-8 inhibited the induced promoter activity. When the two Hox binding sites were mutated, ALK3 or Smad1 linker region no longer activated the transcription. Importantly, Smad1 linker region induced both OPG promoter activity and endogenous OPG protein expression in 2T3 osteoblastic cells. The medium from cells transfected with Smad1 linker region expression plasmid effectively inhibited osteoclastogenesis. Collectively, our data indicate that Hox sites mediate both OPG promoter construct activity and endogenous OPG gene expression in response to BMP stimulation.

Bone remodeling mainly depends on a balance between bone resorption by osteoclasts and bone formation by osteoblasts (1, 2). Cell to cell contact between osteoblasts and osteoclasts is critical for osteoclast differentiation and maturation (3, 4). It is clear that osteoprotegrin (OPG) and osteoprotegrin ligand (OPGL), both produced by osteoblasts, determine the destiny of osteoclast progenitors with the involvement of the osteoclast surface receptor, which is called receptor activator of NF-κB. Thus, OPG and its receptors mediate the communication between osteoblasts and osteoclasts and maintain the balanced bone metabolism (5, 6). OPG, also known as osteoclast differentiation factor, activates osteoclast differentiation. OPG is a secreted receptor of the tumor necrosis factor receptor family without transmembrane domain. It binds directly to OPGL on the osteoblasts/stromal cell surface, inhibiting OPGL-mediated osteoclastogenesis (7, 8). OPG has also been shown to inhibit bone resorption both in vitro and in vivo (9, 10). OPG-deficient mice exhibited severe osteopenia due to the accelerated bone resorption (11, 12). In addition, OPG prevents bone and cartilage loss in a T-cell-dependent arthritis model (13). OPG mRNA was localized mainly to the cartilaginous aspects of developing bone, arteries, skin, and gastrointestinal tract (7), although OPG is also expressed in a variety of tissues, including the kidney, lung, liver, brain, placenta, heart, and hematopoietic cell lines (14, 15).

OPG mRNA expression is regulated by many bone metabolic regulators, such as IL-1 (16), tumor necrosis factors (17), TGF-β (18, 19), vitamin D (20), and prostaglandin E₂ (21) in osteoblastic cells. In particular, bone morphogenetic proteins (BMPs), osteotropic growth factors, significantly induce OPG expression both at the mRNA and the protein level in human fetal osteoblast cells (20). BMPs induce de novo bone formation in postnatal life through the process of intramembranous and endochondral ossification. BMP-2, BMP-4, and BMP-7 are the most potent growth factors that promote new cartilage and bone formation (22–25). The BMPs initiate the cascades of transcription program for mesenchymal stem cell differentiation into osteoblasts and chondrocytes. Studies of the mechanisms of BMP-induced OPG gene transcription should provide us with a better understanding of the roles of BMPs and OPG in skeletal formation and bone remodeling.

BMPs form the largest group in the TGF-β superfamily. The TGF-β signal transduction pathway has been elucidated in recent years (26, 27). Like other members of the superfamily, BMPs bind to two types of transmembrane receptors, known as type I and type II, with serine/threonine kinase activity (28). Upon binding to BMP ligands, type I receptors phosphorylate a family of conserved downstream Smad proteins. Smad1 is the downstream effector of BMP signaling (29, 30). The phosphorylated Smad1 interacts with Smad4, which then translocates into the nucleus and regulates gene transcription by associating with a nuclear transcription factor (31) or by binding directly to DNA (32). Previously, we reported that Smad1 interacts with Hoxc-8, a homeodomain transcription factor, and dislocates Hoxc-8 from its DNA binding element, resulting in the induction of gene expression (33). The interaction domains of Smad1 with Hoxc-8 have been characterized. Overexpression of these domains in osteoblast precursors stimulated osteoblast differentiation-related gene expression and led to mineralized bone matrix formation (34). Hox binding elements widely exist in promoters of osteoblast differentiation marker genes, especially those that rapidly respond to BMP stimulation, such as OPG (20), BMP-4 (35), and osteonectin (36). It appears that the...
interaction between Smad1 and Hox transcription factors may represent the major initiation mechanism of osteoblast differentiation in the BMP signaling pathway.

In the present study, we reported that constitutively active BMP type IA receptor ALK3 (Q233) stimulates OPG promoter activity. By characterization of the OPG promoter, we found that the two Hox binding sites are essential for OPG transcription activation induced by BMP. Most importantly, overexpression of the Smad1 linker region, which interacts with Hoxc-8, induced both OPG promoter activity and endogenous OPG protein expression in 2T3 osteoblastic cells. Finally, the medium from the cells transfected with the Smad1 linker region expression plasmids effectively inhibited OPG-induced osteoclastogenesis.

**EXPERIMENTAL PROCEDURES**

**Plasmid Constructions**—The OPG promoter from −1104 to +59 relative to the transcription start site was amplified by polymerase chain reaction from human genomic DNA and cloned into MluI and BglII sites of the pGL3-basic vector (Promega) to generate a luciferase reporter construct (OPG-1). A deletion series (OPG-2 to −59; OPG-3, −937 to −59; OPG-4, −700 to −59; OPG-5, −564 to −59) was also cloned into MluI and BglII sites of the pGL3-basic vector. OPG-SV40 (−1104 to −624) reporter bearing the two Hox-8 binding sites was constructed using the same strategy but was put into the pGL3-control vector (Promega). The mutants, mOPG-SV40, m1OPG-SV40, and m2OPG-SV40, were generated by replacing both, the first, or the second Hox recognition core sequences TAAT with TCCT (mutated nucleotides are in boldface type), respectively, in the OPG promoter region (−1104 to −624) using polymerase chain reaction. A Smad1 mutant construct (m-Smad1C) was generated by deletion of the Hox-8 interaction domain (amino acids 148–191) of Smad1 (amino acids 148–278).

**Transfection and Luciferase Assay**—C3H10T1/2 mesenchymal cells (5 × 10⁴ cells/12-well plate) were transiently transfected using Tfx-50 reagent (Promega). A total of 0.2 µg of luciferase reporter plasmid (OPG1, OPG-SV40, m1OPG-SV40, m2OPG-SV40, or m2OPG-SV40) and different expression plasmids as indicated. Total DNA was kept constant by the addition of PCDNA3 plasmid. Luciferase activities were assayed 48 h post-transfection using the Dual Luciferase assay kit (Promega) according to the manufacturer's directions. Luciferase values shown in the figures are representative of transfection experiments performed in triplicate. Experiments were repeated at least three independent experiments. We then attempted to localize the BMP response elements by promoter deletion analysis. As shown in Fig. 1, a series of OPG 5′-flanking region deletion constructs were generated. These constructs were transfected in C3H10T1/2 cells and challenged with BMP stimulation. Deletion of the DNA fragment from −1104 to −938 abrogated BMP-induced transcription activity (Fig. 1). Further deletion did not significantly change luciferase activity. Analysis of the DNA sequence of this promoter fragment (−1104 to −938) revealed two putative Hox binding sites, located at −1003 and −943. We therefore examined whether the interaction between Hox-8 and Smad1 is also the transcription mechanism in response to BMP stimulation. Hox-8 was cotransfected with Smad1 and ALK3 in C3H10T1/2 cells. As expected, Hox-8 significantly inhibited BMP-induced OPG transcription (Fig. 1), suggesting that Hox-8 mediates BMP stimulation. This result indicates that the promoter fragment bearing two Hox binding sites may be responsible for BMP-induced transcription. To verify that the promoter region with two Hox sites function as BMP response elements, we cloned the fragment (−1104/−624) containing the two Hox binding sites into the pGL3-control luciferase reporter vector under the control of the SV40 promoter (OPG-SV40; Fig. 2A). When this construct was coexpressed in C3H10T1/2 cells with full-length Smad1 and ALK3 (Q233D), the transcription activity was significantly stimulated (Fig. 2B). Furthermore, the core nucleotides TAAT of these two Hox binding sites were mutated to TCCT, designated as mOPG-SV40. As expected, transfection of the mutant construct completely abolished the Smad1- and ALK3-induced reporter activity and eliminated Hox-8 inhibition in C3H10T1/2 cells. Effects of Hox-8 alone on both wild type and mutant promoter constructs were also examined. Hox-8 sig-

**RESULTS**

**Identification of BMP Response Elements in the OPG Promoter**—BMPs induce OPG gene expression (20). To investigate the mechanisms of the BMP-activated OPG gene transcription, we have cloned an approximately 1-kilobase pair DNA fragment of the OPG promoter (−1104/+59). The OPG promoter was ligated into a luciferase reporter expression vector to examine its responsiveness to BMP stimulation. Transient transfection of this construct in C3H10T1/2 cells with Smad1 and a constitutively active type IA BMP receptor (ALK3) expression plasmid resulted in a significant increase in luciferase activity. We then attempted to localize the BMP response elements by promoter deletion analysis. As shown in Fig. 1, a series of OPG 5′-flanking region deletion constructs were generated. These constructs were transfected in C3H10T1/2 cells and challenged with BMP stimulation. Deletion of the DNA fragment from −1104 to −938 abrogated BMP-induced transcription activity (Fig. 1). Further deletion did not significantly change luciferase activity. Analysis of the DNA sequence of this promoter fragment (−1104 to −938) revealed two putative Hox binding sites, located at −1003 and −943, respectively. We have shown previously that Smad1 interaction with Hox-8 dislodges Hox-8 from its DNA binding element to activate osteopontin gene expression (33). We therefore examined whether the interaction between Hox-8 and Smad1 is also the transcription mechanism in response to BMP stimulation. Hox-8 was cotransfected with Smad1 and ALK3 in C3H10T1/2 cells. As expected, Hox-8 significantly inhibited BMP-induced OPG transcription (Fig. 1), suggesting that Hox-8 mediates BMP stimulation. This result indicates that the promoter fragment bearing two Hox binding sites may be responsible for BMP-induced transcription.
significantly inhibited basal activity in the wild type promoter (Fig. 2B). Two Hox sites were also mutated individually to assess the relative contribution to the BMP stimulation (Fig. 2A). Transfection analysis of these two Hox mutant constructs suggests that mutation of either of the Hox sites abolishes Hoxc-8-mediated inhibition (Fig. 2C). However, the OPG-Hox1 (−1003/−1000) appears to contribute more in response to BMP stimulation. These results indicate that the two Hox binding sites are essential to BMP-induced OPG promoter transcription.

**Hoxc-8 Binds to OPG Promoter Specifically**—To examine whether Hoxc-8 binds to the two consensus Hox binding sites, we performed EMSA. Isotope labeled DNA fragments, containing either one or both of the Hox binding sites, were used in incubation with purified GST-Hoxc-8 (Fig. 3A). When a longer fragment comprising both Hox binding sites was used, Hoxc-8 protein formed a retarded band (Fig. 3B). Thus, Hoxc-8 protein binds to the DNA fragment. Then, two shorter fragments (−1013/−994 and −956/−932), each containing a Hox binding site, were used, respectively.
efficiency to both of the individual Hox binding sites (Fig. 3C, lanes 3 and 8). We have previously shown that Smad1 inhibits Hoxc-8 binding in activating gene transcription (33, 34). The binding specificity of Hoxc-8 to these two Hox sites was examined by both DNA competition studies and Hox site mutation experiments. Each 50-fold unlabeled Hox site displaced the binding of Hoxc-8 to its corresponding Hox site, respectively (Fig. 3C, lanes 4 and 9). As expected, Hoxc-8 binding was abolished when mutated Hox sites were used as probes (Fig. 3C, lanes 5 and 10), in which the core sequence TAAT was mutated into TCCT for both Hox sites. Thus, these results confirm that Hoxc-8 binds to two Hox binding sites of OPG promoter specifically.

**Hoxc-8 Interaction Domain of Smad1 Are Sufficient to Induce OPG Promoter Activation**—We have previously established the BMP-induced gene transcription model (27, 40), in which Smad1 removes Hoxc-8 binding via direct interaction between these two proteins (33). We also mapped the interaction domains for both proteins. The Smad1 linker region is one of the domains (Smad1C, Fig. 4A) that interacts with Hoxc-8 and blocks Hoxc-8 binding to its DNA element (34). The binding of Hoxc-8 to OPG promoter predicts that overexpression of the Smad1 linker region activates OPG promoter transcription. To determine whether such was the case, we co-transfected Smad1C expression plasmids in C3H10T1/2 cells with the OPG promoter construct (OPG1) or the SV40 promoter linked to the two OPG Hox sites (OPG-SV40). Smad1C dramatically stimulates transcription activity for both constructs, and Hoxc-8 inhibits Smad1C-induced transcription activity. Notably, Smad1C with deletion of Smad1 interactin domain did not have such an effect (Fig. 4B). The construct with both mutated Hox sites (mOPG-SV40) or a single Hox site mutation (m1OPG-SV40 and m2OPG-SV40) was transfected in C3H10T1/2 cells. Fig. 4C shows that mutation of one or both Hox sites significantly decreased Smad1C-induced OPG promoter transcription. These results establish that the interaction between Smad1 and Hoxc-8 mediates BMP signals and support our proposed model for BMP-induced gene transcription.

**Smad1-Hoxc-8 Interaction Induces Endogenous OPG Gene Expression**—Having characterized the BMP-induced transcription mechanism for the OPG promoter, we then examined if the OPG promoter analysis-derived transcription mechanism reflects Smad1-mediated endogenous OPG gene expression. C3H10T1/2 cells were transfected with Smad1C expression plasmids. The OPG protein expression levels were measured by Western blot from the Smad1C-transfected 2T3 cells. As seen in Fig. 5A, a 55-kDa band was significantly induced. Another band above 79 kDa appeared later at 32 h after Smad1C transfection, indicating that a large amount of monomer and a small amount of dimer exist in cells. Simonet et al. (7) demonstrated that OPG is synthesized as a 55-kDa monomer within the cell, and the monomers are then converted to disulfide-linked dimers of ~110 kDa. The dimers are then secreted into the medium. The specificity of the OPG band recognized in Western blot is confirmed by completely blocking the band with excess of OPG antigen (Fig. 5B).

Finally, we examined the biological activity of secreted OPG in the medium from Smad1C-transfected cells. Osteoclasts normally differentiate from members of the monocyte-macrophage lineage when exposed to OPGL and M-CSF, but OPG inhibits
The aim of this study is to elucidate the transcription mechanism of BMP-induced OPG gene expression and further explore the interaction between Smad1 and Hoxc-8 as an initial cascade in BMP-induced osteoblast differentiation. We characterized BMP response elements in the OPG promoter. Promoter deletion analysis demonstrates that the OPG promoter fragment bearing two Hox binding sites responds to BMP stimulation. Deletion of the two Hox binding sites abolished BMP-induced OPG promoter activity, and further deletion of other putative Hox binding sites did not change the luciferase activity in response to BMP stimulation. This observation was validated by transfection studies with the construct containing mutations of the two Hox binding sites (Fig. 2B). Furthermore, gel shift assays demonstrate that Hoxc-8 binds to the two Hox sites specifically. There are other putative Hox binding sites identified by the core sequence TAAT in the OPG promoter. However, these Hox sites did not appear to mediate BMP signals in the osteogenic cells. The flanking regions of the “core” are important in determining the specificity of Hox binding, since there are 39 members in the Hox family. It is possible that these Hox binding sites play an essential role in regulating OPG gene expression in different tissues during development.

We then examined whether Smad1 interaction with Hoxc-8 mediates the BMP signals via the two Hox binding sites. We previously showed that the Smad1 linker region interacts with Hoxc-8 to induce osteopontin gene transcription. Overexpression of this interaction domain is sufficient to induce osteoblast differentiation and bone cell formation (34). Similar to osteopontin promoter, transfection of the Smad1 linker region significantly activated OPG promoter transcription in C3H10T1/2 cells, and the induced promoter activity is inhibited by Hoxc-8. Thus, these data suggest the interaction between Smad1 and Hoxc-8 is a general transcription mechanism in the BMP signaling pathway.

The most important functional finding is that the overexpression of Smad1 linker region stimulated endogenous OPG protein expression in C3H10T1/2 cells. The induced OPG protein was secreted into medium, which effectively inhibited osteoclastogenesis. It is known that BMPs play a critical role in bone remodeling and in maintaining the structural integrity of the skeletal system, due to their ability to induce osteoblast differentiation (22–25). OPG, on the other hand, mediates the communication between osteoblast and osteoclast to sustain
bone density. The BMP-induced OPG transcription mechanism revealed in the current study involves Smad1 interaction with Hoxc-8, which is a quick response to BMP stimulation, since it only requires Smad1 phosphorylation. This logically explains the importance of the balance between osteoblast activity and osteoclast activity. The regulation of bone cell differentiation is a very complicated process. OPG is a potent inhibitor of osteoclast activity. The regulation of bone cell differentiation is the importance of the balance between osteoblast activity and osteoclast activity. Their signals are mediated by phosphorylation of BMP receptor-regulated Smads, including Smad1, Smad5, and Smad8. Hoxc-8 has been characterized as the downstream transcription factor of Smad1 in BMP-induced osteoblast differentiation (33), and our data also indicate that Hoxc-8 is specific for the BMP signaling pathway. Data presented in this paper provide additional evidence supporting the hypothesis that the interaction of Smad1 with Hoxc-8 represents the initiation mechanism for BMP-induced osteoblast differentiation. Smad1-Hoxc-8 interaction is the mechanism in activating OPG gene expression, another early marker gene, in response to BMP stimulation. A wide existence of putative Hox binding sites in many other early BMP-responsive genes (35, 36) also suggests the role of Smad1-Hoxc-8 interaction in their BMP-induced gene transcription. However, since Smad1 itself is a sequence-specific transcription factor and there are other BMP-regulated Smads, such as Smad5 and Smad8, an intricate BMP-induced transcription network is anticipated. The BMP-induced transcription cascades will become clear when more BMP downstream transcription factors are identified and more transcription mechanisms are characterized from BMP-regulated genes.

Acknowledgments—We are grateful to J. Wrana for kindly providing the constitutively active BMP type IA (ALK3) receptor expression vector, R. Derynick for human Smad1 cDNA clones, and H. Le Mouellec for Hoxc-8 cDNA expression vectors.

REFERENCES
1. Manolages, S. C., and Jilka, R. L. (1995) Endocr. Rev. 16, 80–89
2. Horowitz, M. C. (1993) Science 260, 626–627
3. Suda, T., Takahashi, N., and Martin, T. J. (1992) Endocr. Rev. 13, 66–80
4. Chambers, T. J., Owens, J. M., Hattersley, G., Jat, P. S., and Noble, M. D. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5578–5582
5. Yasuda, H., Shima, N., Nakagawa, N., Yamaguchi, K., Kinosaki, M., Mochizuki, S., Tomoyasu, A., Yano, K., Goto, M., Murakami, A., Tsuda, E., Morinaga, T., Higashio, K., Udagawa, N., Takahashi, N., and Suda, T. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 3597–3602
6. Lacey, D. L., Timms, E., Tan, H. L., Kelley, M. J., Dunstan, C. R., Burgess, T., Eliott, R., Colombero, A., Ellitt, G., Seully, S., Hau, H., Sullivan, J., Hawkins, N., Davy, E., Capparelli, C., Eli, A., Qian, Y. X., Kaufman, S., Saras, I., Shalhub, V., Senaldi, G., Guo, J., Delaney, J., and Boyle, W. J. (1998) Cell 93, 165–176
7. Simonet, W. S., Lacey, D. L., Dunstan, C. R., Kelley, M., Chang, M. S., Luthy, R., Nguyen, H. Q., Wooden, S., Bennett, L., Boone, T., Shimamoto, G., DeRose, M., Elliott, R., Colombero, A., Tan, H. L., Prail, G., Sullivan, J., Davy, E., Bucay, N., Renshaw-Gegg, L., Hughes, T. M., Hill, D., Pattison, W., Campbell, P., Sander, S., Van, G., Tarpley, J., Derby, P., Lee, R., and Boyle, W. J. (1997) Cell 89, 309–319
8. Kong, Y-Y., Yoshida, H., Saras, I., Tan, H. L., Timms, E., Capparelli, C., Morony, S., Oliveira-dos-Santos, A. J., Van, G., Irie, A., Khoo, W., Wakeham, A., Dunstan, C. R., Lacey, D. L., Mak, T. W., Boyle, W. J., and Penninger, J. M. (1999) Nature 397, 315–323
9. Hakeda, Y., Kobayashi, Y., Yamaguchi, K., Yasuda, H., Tsuda, E., Higashio, K., Miyata, T., and Kumegawa, M. (1998) Biochem. Biophys. Res. Commun. 251, 796–801

FIG. 6. Osteoclast formation from bone marrow precursors was inhibited by the media from Smad1C transfected 2T3 cells. Mouse bone marrow macrophages (BMMs) were cultured with (A, C, and D) or without (B) M-CSF (10 ng/ml) and GST-OPGL (50 ng/ml). Media from 2T3 cells transfected with PCDNA3 empty vector (C) or Smad1C construct (D) were added to M-CSF-stimulated BMMs (media from transfected 2T3 cells/bone marrow cell media = 1:3). Multinucleated osteoclasts were shown by TRAP staining. Upper panels, direct scan of the stained 12-well plate; bottom panels, representative photomicrographs (×10 objective) of TRAP-stained cultures.
10. Tsuda, E., Goto, M., Mochizuki, S., Yano, K., Kobayashi, F., Morinaga, T., and Higashio, K. (1997) Biochem. Biophys. Res. Commun. 234, 137–142

11. Bocay, N., Sarosi, I., Dunstan, C. R., Maroney, S., Tarpley, J., Capparelli, C., Scully, S., Tan, H. L., Xu, W., Lacey, D. L., Boyle, W. J., and Simonet, W. S. (1998) Genes Dev. 12, 1260–1268

12. Miura, A., Amizuka, N., Irie, K., Murakami, A., Fujioe, N., Kanno, T., Sato, Y., Nakagawa, N., Yasuda, H., Mochizuki, S., Gomibuchi, T., Yano, K., Shima, N., Washida, N., Tsuda, E., Morinaga, T., and Higashio, K., and Ozawa, H. (1998) Biochem. Biophys. Res. Commun. 247, 610–615

13. Kong, Y.-Y., Peige, U., Sarosi, I., Belon, B., Tafuri, A., Morony, S., Capparelli, C., Li, J., Elliott, R., McCabe, S., Wong, T., Catapano, G., Morán, E., Bogoch, E., Van, G., Nguyen, L., Ohashi, P., Lacey, D., Fish, E., Boyle, W., and Penninger, J. (1999) Nature 402, 304–309

14. Yasuda, H., Shima, N., Nakagawa, N., Mochizuki, S. I., Yano, K., Fujioe, N., Sato, Y., Goto, M., Yamaguchi, K., Kuriyama, M., Kanno, T., Murakami, A., Tsuda, E., Morinaga, T., and Higashio, K. (1998) Endocrinology 139, 1329–1337

15. Kwon, B. S., Wang, S., Udagawa, N., Haridas, V., Lee, Z. H., Kim, K. K., Oh, K. O., Green, J., Li, Y., Su, J., Gentz, R., and Aggarwal, B. B. (1998) Biochem. Biophys. Res. Commun. 250, 776–781

16. Vidal, O. N., Sjogren, K., Eriksson, B. I., Ljunghall, S., and Ohlsson, C. (1998) Biochem. Biophys. Res. Commun. 246, 696–700

17. Brandstrom, H., Jonsson, K. B., Vidal, O., Ljunghall, S., Ohlsson, C., and Ljunghgren, O. (1998) Biochem. Biophys. Res. Commun. 248, 454–457

18. Takai, H., Kanematsu, M., Yano, K., Tsuda, E., Higashio, K., Ikeda, K., Watanabe, K., and Yamada, Y. (1998) J. Biol. Chem. 273, 27091–27096

19. Murakami, T., Yamamoto, M., Yamamoto, M., Ota, K., Nishikawa, M., Nogata, N., Motoyoshi, K., and Akatsu, T. (1998) Biochem. Biophys. Res. Commun. 252, 747–752

20. Hoyer, W. C., Dunstan, C. R., Spelsberg, T. C., Riggs, B. L., and Kholas, S. (1999) Biochem. Biophys. Res. Commun. 250, 776–781

21. Brandstrom, H., Jonsson, K. B., Ohlsson, C., Vidal, O., Ljunghall, S., and Ljunghgren, O. (1998) Biochem. Biophys. Res. Commun. 247, 338–341

22. Schmitt, J. M., Hwang, K., Shelley, R. W., and Jeffery, O. H. (1999) J. Orthop. Res. 17, 269–278

23. Wang, E. A., Rosend, V., D’Alessandro, J. S., Bauduy, M., Cordes, P., Harada, T., Israel, D. I., Hewick, R. M., Kerns, K. M., and LaPan, P. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 9281–9288

24. Michel, K. F., Hallewell-Dilke, K., Zheng, M. H., Namba, N., Lam, J., Novack, D., Feng, X., Ross, F. P., Hynes, R. O., and Teitelbaum, S. L. (2000) J. Clin. Invest. 105, 433–440

25. Massague, J. (1998) Annu. Rev. Biochem. 67, 753–791

26. Wrana, J. F. (2000) Cell 100, 189–192

27. Kawabata, M., Inoue, H., Hanyu, A., Imamura, T., and Miyazono, K. (1998) EMBO J. 17, 4056–4065

28. Hoodless, P. A., Haeryr, T., Abdollah, S., Stapleton, M., O’Connor, M. B., Attisano, L., and Wrana, J. L. (1996) Cell 85, 489–500

29. Kretzschmar, M., Liu, F., Hata, A., Doody, J., and Massague, J. (1997) Genes Dev. 11, 984–995

30. Verschueren, K., Remacle, J. E., Collart, C., Kraft, H., Baker, B. S., Tylzanowski, P., Nelless, L., Wuytens, G., Su, M. T., Bodmer, R., Smith, J. C., and Huybrechts, D. (1999) J. Biol. Chem. 274, 20499–20496

31. Kim, J., Johnson, K., Chen, H. J., Columbus, S. E. (1995) J. Biol. Chem. 270, 9281–9288

32. Stone, R. G., Jr, Findlay, D. M., Martin, T. J., and Ng, K. W. (1999) J. Cell. Physiol. 175, 112–119

33. Stern, J. M., Murata, Y., Kim, H. G., Bennett, S. E., Templeton, D. J., and Horowitz, J. M. (1995) J. Biol. Chem. 270, 9281–9288

34. Bizzarri, C., Shioi, A., Teitelbaum, S. L., Ohara, J., Harwalkar, V. A. Erdman, J., Lacey, D. L., and Civitelli, R. (1994) J. Biol. Chem. 269, 13817–13824

35. McHugh, K. P., Brown, D. T., Zheng, M. H., Namba, N., Lam, J., Novack, D., Feng, X., Ross, F. P., Hynes, R. O., and Teitelbaum, S. L. (2000) J. Cell. Biol. 150, 433–440

36. Massague, J., and Wotton, D. (2000) EMBO J. 19, 1745–1754
Transcriptional Mechanisms of Bone Morphogenetic Protein-induced Osteoprotegrin Gene Expression
Mei Wan, Xingming Shi, Xu Feng and Xu Cao

J. Biol. Chem. 2001, 276:10119-10125.
doi: 10.1074/jbc.M006918200 originally published online January 3, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M006918200

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

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

This article cites 40 references, 15 of which can be accessed free at http://www.jbc.org/content/276/13/10119.full.html#ref-list-1