Vol. 204, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 14, 1994 Pages 203-209

MOLECULAR CLONING OF RAT BONE MORPHOGENETIC PROTEIN (BMP) TYPE IA RECEPTOR AND ITS EXPRESSION DURING ECTOPIC BONE FORMATION INDUCED BY BMP+

Kohsuke Takeda[1,2], Shinichiro Oida[1,*], Hidenori Ichijo[4], Tadahiro Iimura[1], Yutaka Maruoka[3], Teruo Amagasa[2], and Satoshi Sasaki[1]

[1]Department of Biochemistry, [2]First and [3]Second Departments of Oral and Maxillofacial Surgery, and [4]Department of Oral Pathology, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo 113, Japan

Received September 1, 1994

Summary: A cDNA for the rat bone morphogenetic protein (BMP) type IA receptor (BMPR-IA) was isolated from a dental pulp cell cDNA library. The rat BMPR-IA cDNA encodes a protein of 532 amino acids with a single transmembrane domain and a putative serine/threonine kinase domain. The overall amino acid sequence identity between the rat and human BMPR-IA was 97 %. Reverse transcriptase-polymerase chain reaction analysis revealed that BMPR-IA mRNA was highly expressed in the BMP-induced bone forming tissues throughout the stages tested. © 1994 Academic Press, Inc.

Bone morphogenetic proteins (BMPs), originally identified in the extract of demineralized bone, induce endochondral bone formation by their intramuscular or subcutaneous implantation (reviewed in Refs. 1,2). To date, eight different BMP polypeptides termed BMP-1 to BMP-8 have been isolated (3-6). Based on the homology of the primary amino acid sequences, seven out of the eight BMPs (BMP-2 to -8) have been shown to be the members of a transforming growth factor-ß (TGF-ß) superfamily, a large family of structurally related signaling proteins with diverse activities on cell growth and differentiation (reviewed in Ref. 7).

Members of the TGF-ß superfamily, including TGF-ßs, activins and BMPs, exert their effects by binding to the cell surface receptors. Type I (50-55 kDa) and type II (70-80 kDa) receptors for TGF-ßs and activins are transmembrane serine/threonine kinases, which signal through formation of heteromeric receptor complexes on ligand binding (8-

+The nucleotide sequence data reported in this communication will appear in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databases with the following accession number D38082.

*To whom correspondence should be addressed.

0006-291X/94 $5.00
Copyright © 1994 by Academic Press, Inc.
All rights of reproduction in any form reserved.

11). Recently, a series of novel serine/threonine kinase receptors, termed activin receptor-like kinases (ALK)-1 to -6, have been identified (11-13). Among these receptors, ALK-3/BMPR-IA and ALK-6/BMPR-IB were identified as type I receptors for BMP-4 and osteogenic protein-1 (OP-1)/BMP-7 (17), whereas ALK-2/ActR-I which was characterized as a type I receptor for activin (13-16) was shown to serve as a type I receptor for OP-1/BMP-7 as well (17).

In this communication, we show the molecular cloning of rat BMPR-IA and investigate the expression of BMPR-IA mRNA during ectopic bone formation induced by BMP.

MATERIALS AND METHODS

Preparation of BMP. BMP was partially purified by the method as described previously (18). In brief, powdered bovine bone was decalcified with 0.6N HCl, solubilized with 4M guanidine hydrochloride (Gdn. HCl), dialyzed against distilled water, and lyophilized (G-ext). The insoluble residue of the bone matrix in 4M Gdn.HCl was separated by centrifugation and lyophilized (G-res). G-ext was subjected to gel filtration through a Sephacryl S-200 column eluted with 4M Gdn. HCl/50mM Tris HCl, pH7.4. BMP-containing fraction (1.5mg of total protein amount) was mixed with 15 mg of G-res, and used for the *in vivo* implantation.

Implantation of BMP and Preparation of RNA. BMP was implanted into subcutaneous muscle layer of six-week-old Wistar strain rats. Tissues surrounding the implants were harvested at intervals of 3, 6 and 9 days after implantation; total RNA was prepared from the extracted tissue by the acid guanidinium thiocyanate-phenol-chloroform method (19). Poly(A)+RNA was isolated by binding to oligo(dT)-cellulose (Type 3, Collaborative Research).

Polymerase Chain Reaction (PCR). First-strand cDNA was reverse transcribed from 1 μg of poly(A)+RNA using the SuperScript preamplification system (Gibco BRL) and used as a template for PCR. Nucleotide sequences 458-478 and 885-905 of human ALK-3/BMPR-IA (12) were used for PCR primers. PCR conditions were as follows; 5 cycles of 94°C (1 min), 50°C (1 min) and 72°C (1 min), and 25 cycles of 94°C (30 sec), 55°C (30 sec) and 72°C (30 sec), followed by 10 min at 72°C. The PCR products were subcloned into pBluescript SK(+) (Stratagene) and sequenced by the BcaBEST dideoxy sequencing kit (Takara) and the A.L.F. sequencing (Pharmacia).

Construction of cDNA Library and Cloning of Rat BMPR-IA cDNA. Poly(A)+ RNA was extracted from a rat dental pulp cell line RPC-C2A(20). An oligo(dT)-primed cDNA library with 1 x 10^6 independent clones was prepared by the Uni-ZAP XR/Gigapack II Gold Cloning Kit (Stratagene). Approximately 6 x 10^5 clones of the RPC-C2A cDNA library were screened with the PCR probe [^{32}P]-labeled by the Ready-To-Go DNA labeling kit (Pharmacia). Hybridization to duplicate nylon filters was performed as described previously (21). Two positive clones were isolated and rescued into pBluescript SK(-). Nucleotide sequencing was performed on both strands.

Southern Blot Analysis of the PCR Products. Five microliters of the PCR products were electrophoresed on a 1.0% agarose gel and blotted onto a nylon filter (Zeta-Probe, Bio-Rad). The filter was probed with radiolabeled rat BMPR-IA cDNA. As an internal control, southern blot analysis of G3PDH was performed using G3PDH Amplimer Set and cDNA (Clontech).

RESULTS AND DISCUSSION

Cloning of Rat BMPR-IA cDNA. Our previous histologic observations on *in vivo* implantation of BMP have revealed that administration of BMP into rat subcutaneous muscle layer was followed sequentially by infiltration of undifferentiated mesenchymal cells, cartilage formation and endochondral bone formation 3, 6 and 9 days after implantation, respectively (22). These observations strongly suggested that BMP receptors are expressed at the site of the ectopic bone formation induced by BMP. To elucidate the mechanism of the BMP-induced bone formation, we first attempted to isolate a rat BMP receptor cDNA from the BMP-implanted tissue. Reverse transcriptase (RT)-PCR with primers based on the sequence of human BMPR-IA (12) was performed using a template cDNA pool of the BMP-implanted tissue (3 days after implantation). A 448bp PCR fragment with 89% nucleotide sequence identity to human BMPR-IA was obtained. The predicted amino acid sequence showed 95 % identity to human BMPR-IA, indicating that the amplified fragment encodes the rat BMPR-IA. To isolate a full-length cDNA for the rat BMPR-IA, cDNA library prepared from a rat dental pulp cell line RPC-C2A was then screened using the PCR fragment as a probe; two cDNA clones termed P1144 and P1146 were obtained.

Structure of the Rat BMPR-IA. cDNA sequencing analysis of clone P1144 yielded a 2620 bp fragment consisting of an open reading frame of 1596 bp, flanked by a 5' untranslated sequence of 81 bp, and a 3' untranslated sequence of 942 bp. The nucleotide sequence and the predicted amino acid sequence of P1144 are shown in Fig. 1. No poly(A) tail was found in the 3' untranslated sequence of P1144, suggesting that the cDNA was derived from internal priming. An ATG codon (nucleotides 82-84) fitting with the rules for the translational initiation (23) was found in the 5' part of the open reading frame following an in-frame stop codon (nucleotides 43-45). P1146 clone with an insert of 2698 bp lacked 326 nucleotides of the 5' corresponding sequence of P1144, whereas the sequence extended at the 3' end with 404 bp containing a poly(A) tail (data not shown). The open reading frame sequence of P1144 encoded 532 amino acid residues containing two hydrophobic regions at the amino acids 1-23 and 153-176 , which correspond to a signal peptide and a transmembrane domain, respectively (12). Thus, the mature rat BMPR-IA protein is predicted to be a 509 amino acid

```
CGTTCAGTAAGGCCGTTTACTTCAGTGAAACAGCAGGACCAGTAATCAAGGTGGCCCGGACAGGACACGTGCGAATTGGACAATGACTCAGCTATACACTTACATCAGATTACTGGGAGC   120
                                                                  M  T  Q  L  Y  T  Y  I  R  L  L  G  A                    13

CTGTCTGTTCATCATTTCTCATGTTCAAGGGCAGAATCTAGATAGTATGCTCCATGGTACTGGTATGAAATCAGACGTGGACCAGAAGAAGCCGGAAAATGGAGTGACGTTAGCACCAGA   240
C  L  F  I  I  S  H  V  Q  G  Q  N  L  D  S  M  L  H  G  T  G  M  K  S  D  V  D  Q  K  K  P  E  N  G  V  T  L  A  P  E    53

GGACACCTTACCTTTCTTAAAATGCTATTGCTCAGGACACTGCCCAGATGACGCTATTAATAACACATGCATAACTAATGGCCATTGCTTTGCCATTATAGAAGAAGATGATCAGGGAGA   360
D  T  L  P  F  L  K  C  Y  C  S  G  H  C  P  D  D  A  I  N  N  T  C  I  T  N  G  H  C  F  A  I  I  E  E  D  D  Q  G  E    93

AACCACGTTAACTTCTCGGTGTATGAAGTATGAAGGCTCTGATTTTCAATGCAAGGATTCACCAAAAGCCCAGCTACGCAGGACAATAGAATGTTGTCGGACCAATTTGTGCAACCAATA   480
T  T  L  T  S  G  C  M  K  Y  E  G  S  D  F  Q  C  K  D  S  P  K  A  Q  L  R  R  T  I  E  C  C  R  T  N  L  C  N  Q  Y   133

TTTGCAGCCTACACTGCCCCCTGTCGTTATAGGCCCATTCTTTGATGGCAGCGTCCGATGGCTGGCTGTGCTCATCTCTATGGCTGTCTGTATTGTCGCCATGATCGTCTTCTCCAGCTG   600
L  Q  P  T  L  P  P  V  V  I  G  P  F  F  D  G  S  V  R  W  L  A  V  L  I  S  M  A  V  C  I  V  A  M  I  V  F  S  S  C   173

CTTCTGTTACAAACATTACTGTAAGAGTATCTCAAGCAGAGGTCGTTACAACCGTGACTTGGAACAGGATGAAGCATTTATTCCAGTAGGAGAATCACTGAAAGACCTGATTGACCAGTC   720
F  C  Y  K  H  Y  C  K  S  I  S  S  R  G  R  Y  N  R  D  L  E  Q  D  E  A  F  I  P  V  G  E  S  L  K  D  L  I  D  Q  S   213

ACAAAGCTCTGGTAGTGGATCTGGATTACCTTTATTGGTTCAGCGAACTATTGCCAAACAGATTCAGATGGTTCGGCAGGTTGGTAAAGGCCGGTATGGAGAAGTATGGATGGGTAAATG   840
Q  S  S  G  S  G  S  G  L  P  L  L  V  Q  R  T  I  A  K  Q  I  Q  M  V  R  Q  V  G  K  G  R  Y  G  E  V  W  M  G  K  W   253

GCGTGGTGAAAAAGTGGCTGTCAAAGTATTTTTTACCACTGAAGAAGCTAGCTGGTTTAGAGAAACAGAAATCTACCAGACGGTGTTAATGCGTCATGAAAATATACTTGGTTTTATAGC   960
R  G  E  K  V  A  V  K  V  F  F  T  T  E  E  A  S  W  F  R  E  T  E  I  Y  Q  T  V  L  M  R  H  E  N  I  L  G  F  I  A   293

TGCAGACATTAAAGGCACCGGTTCCTGGACTCAGCTGTATTTGATTACTGATTACCATGAGAATGGGTCTCTCTATGACTTCCTGAAATGTGCCACCCTGGACACCAGAGCCCTACTCAA  1080
A  D  I  K  G  T  G  S  W  T  Q  L  Y  L  I  T  D  Y  H  E  N  G  S  L  Y  D  F  L  K  C  A  T  L  D  T  R  A  L  L  K   333

GTTAGCTTATTCTGCTGCCTGTGGTCTGTGCCACCTCCACACAGAAATTTATGGCACGCAAGGCAAGCCTGCAATTGCTCATCGAGACCTGAAGAGCAAAAACATCCTTATTAAGAAAAA  1200
L  A  Y  S  A  A  C  G  L  C  H  L  H  T  E  I  Y  G  T  Q  G  K  P  A  I  A  H  R  D  L  K  S  K  N  I  L  I  K  K  N   373

TGGTAGTTGCTGTATTGCTGACCTGGGCCTAGCTGTTAAATTCAACAGTGACACAAATGAAGTTGACATACCCTTGAACACCAGGGTGGGCACCAGGCGGTACATGGCTCCAGAAGTGCT  1320
G  S  C  C  I  A  D  L  G  L  A  V  K  F  N  S  D  T  N  E  V  D  I  P  L  N  T  R  V  G  T  R  R  Y  M  A  P  E  V  L   413

GGACGAGAGCCTGAGTAAAAACCATTTCCAGCCCTACATCATGGCTGACATCTACAGCTTTGGTTTGATCATTTGGGAGATGGCCCGTCGCTGTATTACAGGAGGAATCGTGGAGGAATA  1440
D  E  S  L  S  K  N  H  F  Q  P  Y  I  M  A  D  I  Y  S  F  G  L  I  I  W  E  M  A  R  R  C  I  T  G  G  I  V  E  E  Y   453

TCAATTACCATATTACAACATGGTGCCTAGTGACCCATCTTATGAAGACATGCGTGAGGTCGTGTGTGTGAAACGCTTGCGGCCAATCGTCTCTAACCGCTGGAACAGTGATGAATGTCT  1560
Q  L  P  Y  Y  N  M  V  P  S  D  P  S  Y  E  D  M  R  E  V  V  C  V  K  R  L  R  P  I  V  S  N  R  W  N  S  D  E  C  L   493

TCGAGCCGTTTTGAAGCTGATGTCAGAATGCTGGGCCCATAATCCAGCATCCAGACTCACAGCTTTGAGAATCAAGAAGACGCTCGCAAAGATGGTTGAATCCCAGGATGTAAAGATTTG  1680
R  A  V  L  K  L  M  S  E  C  W  A  H  N  P  A  S  R  L  T  A  L  R  I  K  K  T  L  A  K  M  V  E  S  Q  D  V  K  I  *   532

ACAAACAGTTTTGAGAAAGAATTTAGACTGCAAGAAATTCACCCGAGGAAGGGTGGAGTTAGCATGGACTAGGATGTCGGCTTGGTTTCCAGACTCTCTCCTCTACATCTTCACAGGCTG  1800
CTAACAGTAAACTTTCAGGACTCTGCAGAATGCAGGGTTGGAGCTTCAGACATAGGACTTCAGACATGCTGTTCTTTGCGTATGGACAGCTTTGTTTTAAATGTGGGCTTTTGATGCCTT  1920
TTTGGTTTTTATGAATTGCATCAAGACTCCAATCCTGATAAGAAGTCTCTGGTCAAACTCTGGTTACTCACTATCCTGTCCATAAAGTGGTGCTTTCTGTGAAAGCCTTAAGGAAATTAG  2040
TGAGCTCAGCAGAGATGGAGAAAGGCATATTTGCCCTCTACAGAGAAAATATCTGTCTGTGTTCTGTCTCTGTAAACAGCCTGGACTATGATCTCTTTGGGATGCTGCCTGGTTGATGAT  2160
GGTGCATCATGCCTCTGATATGCATACCAGACTTCCTCTGCTGCCATGGGCTTACAAGACAAGAATGTGAAGGTTGCACAGGACGGTATTTGTGGCCAGTGGTTTAAATATGCAATATCT  2280
AATCGACATTCGCCAATCTCATAAAAGCCATCTACCTTGTAACTGAAGTAACTTCTCTACCAACTTTATTTTTAGCATAATAGTTGTAAAGGCCAAACTATGTATAAAGTGTCCATAGAC  2400
TCGAACTGTTTTCCTCCAGTCACCATTTTGTTTTCCTTTTGGTAATTATTTTTGTTATATAATTCCTCCTATCCAGAATTGGCGCTCACTGTCTTGAACCATACTTTGAAAGAAATGCCT  2520
CTTCCTGGAGTCTGCCTTACTGCATCTGATCACCATGTGCATACCTCTGATCAAATTCTGGAGTCTTTGTTCTCGGTACCTCTTAAAAAGGGAAATTGTG                    2620
```

Fig. 1. The nucleotide sequence and predicted amino acid sequence of rat BMPR-IA. The N-terminal hydrophobic signal peptide and the putative transmembrane domain are underlined. The site for potential N-linked glycosylation is double-underlined. Cysteine residues in the extracellular domain are boxed. The borders of the kinase domain are indicated by arrows.

transmembrane protein with a 129 amino acid extracellular domain, and a 356 amino acid intracellular domain.

Comparison of the Rat and Human BMPR-IA (Fig. 2). The overall amino acid sequence identity of the rat BMPR-IA with the human counterpart (12) was 97%. One potential N-linked glycosylation site and ten cysteine residues in the extracellular domain

```
RAT   MTQLYTYIRL LGACLFIISH VQGQNLDSML HGTGMKSDVD QKKPENGVTL APEDTLPFLK   60
HUMAN      I        Y     R                      S     S

RAT   CYCSGHCPDD AINNTCITNG HCFAIIEEDD QGETTLTSGC MKYEGSDFQC KDSPKAQLRR  120
HUMAN                                          A

RAT   TIECCRTNLC NQYLQPTLPP VVIGPFFDGS VRWLAVLISM AVCIVAMIVF SSCFCYKHYC  180
HUMAN                                  I  VL         I   I

RAT   KSISSRGRYN RDLEQDEAFI PVGESLKDLI DQSQSSGSGS GLPLLVQRTI AKQIQMVRQV  240
HUMAN       R

RAT   GKGRYGEVWM GKWRGEKVAV KVFFTTEEAS WFRETEIYQT VLMRHENILG FIAADIKGTG  300
HUMAN

RAT   SWTQLYLITD YHENGSLYDF LKCATLDTRA LLKLAYSAAC GLCHLHTEIY GTQGKPAIAH  360
HUMAN

RAT   RDLKSKNILI KKNGSCCIAD LGLAVKFNSD TNEVDIPLNT RVGTKRYMAP EVLDESLSKN  420
HUMAN                                          V         K           N

RAT   HFQPYIMADI YSFGLIIWEM ARRCITGGIV EEYQLPYYNM VPSDPSYEDM REVVCVKRLR  480
HUMAN

RAT   PIVSNRWNSD ECLRAVLKLM SECWAHNPAS RLTALRIKKT LAKMVESQDV KI          532
HUMAN
```

Fig. 2. Comparison of the amino acid sequences of the rat and human BMPR-IA. In the human sequence, only amino acids which are different from those of rat are shown. Numbers indicate the amino acid residues of the rat sequence. The hydrophobic signal peptide and transmembrane domain are overlined. The conserved cysteine residues in the extracellular domain (*) and the potential glycosylation site (#) are indicated. The kinase domain is underlined.

of the mature protein were all conserved. Only three amino acid substitutions, all located in subdomain VIII (24), were found in the intracellular serine/threonine kinase domain, resulting in 99% amino acid sequence identity.

Expression of BMPR-IA mRNA during Ectopic Bone Formation. To investigate the expression of BMPR-IA mRNA in the BMP-implanted tissue, we performed RT-PCR-based analysis using cDNAs from the BMP-implanted tissue extracted 3, 6 and 9 days after implantation. cDNA from rat skeletal muscle was also used as a control. Fig. 3 shows the results of southern blot analysis of the RT-PCR products. Expression of BMPR-IA mRNA was detected at similar levels in all stages tested, whereas much lower level of expression was observed in skeletal muscle (Fig. 3, lane M). These results suggest that a relatively high level of BMPR-IA mRNA expression was maintained throughout the stages of BMP-induced ectopic bone formation.

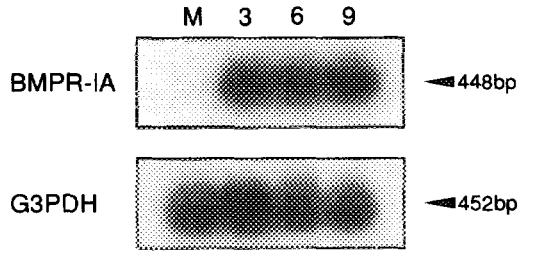

Fig. 3. Expression of BMPR-IA mRNA during ectopic bone formation induced by BMP. The BMP-implanted tissues and skeletal muscle were examined for expression of BMPR-IA mRNA by southern blot analysis following RT-PCR. G3PDH was used as an internal control. The size of the PCR products is indicated on the right. Lane M indicates skeletal muscle; lanes 3, 6 and 9 indicate the BMP-implanted tissues 3, 6 and 9 days after implantation, respectively.

BMP is thought to induce bone formation through sequential multistep cascades consisting of chemotaxis of progenitor cells, mitosis of mesenchymal cells, and differentiation of cartilage and bone (reviewed in Ref. 25). However, little is known about the primary target cells or tissues of BMP and the molecular interactions between BMP and the receptors. Recent cDNA cloning and binding experiments of a series of mammalian receptor serine/threonine kinases demonstrated that BMP-4 and OP-1/BMP-7 bind ALK-3/BMPR-IA and ALK-6/BMPR-IB, whereas OP-1/BMP-7 binds type I receptor for activin as well (17); however, it is still unclear whether these molecules are functinal receptors in mediating specific signals for the ectopic bone formation. Our findings that one of the BMP receptors, BMPR-IA, are highly conserved between speices and are expressed during the BMP-induced bone formation may suggest that BMPR-IA plays important roles for the ectopic bone formation.

To understand the mechanism of BMP action(s), it would be of importance to identify the cells that express BMP receptors during the BMP-induced ectopic bone formation. *In situ* hybridization analysis of BMP-implanted tissues with the rat BMPR-IA cDNA probe are currently being performed in our laboratory.

Acknowledgments: We thank Dr. Kohei Miyazono for valuable discussion. We also thank Dr. Shohei Kasugai for RPC-C2A cells.

REFERENCES

1. Rosen, V. and Thies, R.S. (1992) *Trend Genet.* **8**, 97-102.
2. Wozney, J.M. (1992) *Mol. Reprod. Dev.* **32**, 160-167.

3. Wozney, J.M., Rosen, V., Celeste, A.J., Mitsock, L.M., Whitters, M.J., Kriz, R.W., Hewick, R.M., and Wang, E.A. (1988) *Science* **242**, 1528-1534.
4. Celeste, A.J., Iannazzi, J.A., Taylor, R.C., Hewick, R.M., Rosen, V., Wang, E.A., and Wozney, J.M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9843-9847.
5. Özkaynak, E., Rueger, D.C., Drier, E.A., Corbett, C., Ridge, R.J., Sampath, T.K.,and Oppermann, H. (1990) *EMBO J.* **9**, 2085-2093.
6. Özkaynak, E., Schnegelsberg, P.N.J., Jin, D.F., Clifford, G.M., Warren, F.D., Drier, E.A., and Oppermann, H. (1992) *J. Biol. Chem.* **267**, 25220-25227.
7. Kingsley, D.M. (1994) *Genes & Dev.* **8**, 133-146.
8. Mathews, L.S., and Vale W.W. (1991) *Cell* **65**, 973-982.
9. Attisano, L., Wrana, J.L., Cheifetz, S., and Massagué, J. (1992) *Cell* **68**, 97-108.
10. Lin, H.Y., Wang, X.-F., Ng-Eaton, E., Weinberg, R.A., and Lodish, H.F. (1992) *Cell* **68**, 775-785. Erratum: *Cell* **70**(6).
11. Franzén, P., ten Dijke, P., Ichijo, H., Yamashita, H., Schulz, P., Heldin, C.-H., and Miyazono, K. (1993) *Cell* **75**, 681-692.
12. ten Dijke, P., Ichijo, H., Franzén, P., Schulz, P., Saras, J., Toyoshima, H., Heldin, C.-H., and Miyazono, K. (1993) *Oncogene* **8**, 2879-2887.
13. ten Dijke, P., Yamashita, H., Ichijo, H., Franzén, P., Laiho, M., Miyazono, K., and Heldin, C.-H. (1994) *Science* **264**, 101-104.
14. Ebner, R., Chen, R.-H., Lawler, S., Zioncheck, T.F., and Derynck, R. (1993) *Science* **262**, 900-902.
15. Attisano, L., Carcamo, J., Ventura, F., Weis, F.M.B., Massagué, J., and Wrana, J.L. (1993) *Cell* **75**, 671-680.
16. Tsuchida, K., Mathews, L.S., and Vale, W.W. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 11242-11246.
17. ten Dijke, P., Yamashita, H., Sampath, T.K., Reddi, A.H., Estevez, M., Riddle, D.L., Ichijo, H., Heldin, C.-H., and Miyazono, K. (1994) *J. Biol. Chem.* **269**, 16985-16988.
18. Harada, K., Oida, S., and Sasaki, S. (1988) *Bone* **9**, 177-183.
19. Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* **162**, 156-159.
20. Kasugai, S., Adachi, M., and Ogura, H. (1988) *Archs oral Biol.* **33**, 887-891.
21. Takeda, K., Oida, S., Goseki, M., Iimura, T., Maruoka, Y., Amagasa, T., and Sasaki, S. (1994) *Bone* **15**, 467-470.
22. Iimura, T., Oida, S., Takeda, K., Maruoka, Y., and Sasaki, S. (1994) *Biochem. Biophys. Res. Commun.* **201**, 980-987.
23. Kozak, M. (1987) *Nucleic Acids Res.* **15**, 8125-8148.
24. Hanks, S.K., Quinn, A.M., and Hunter, T. (1988) *Science* **241**, 42-52.
25. Reddi, A.H. and Cunningham, N.S. (1993) *J. Bone Min. Res.* **8**, S499-502.