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Cloning and characterization of a human type II receptor for bone morphogenetic proteins

[osteogenic protein/transforming growth factor β superfamily/serine(threonine) kinase receptor/cDNA cloning]

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ABSTRACT **Bone morphogenetic proteins (BMPs) are members of the transforming growth factor β superfamily. Several members of this family have been shown to transduce their signals through binding to type I and type II serine-(threonine) kinase receptors. Here we report the cDNA cloning and characterization of a human type II receptor for BMPs (BMPR-II), which is distantly related to DAF-4, a BMP type II receptor from *Caenorhabditis elegans*. In transfected COS-1 cells, osteogenic protein (OP)-1/BMP-7, and less efficiently BMP-4, bound to BMPR-II. BMPR-II bound ligands only weakly alone, but the binding was facilitated by the presence of previously identified type I receptors for BMPs. Binding of OP-1/BMP-7 to BMPR-II was also observed in nontransfected cell lines. Moreover, a transcriptional activation signal was transduced by BMPR-II in the presence of type I receptors after stimulation by OP-1/BMP-7.**

Bone morphogenetic proteins (BMPs) are a family of proteins that induce bone formation at extraskeletal sites *in vivo* (reviewed in refs. 1–3). BMPs act on osteoblasts and chondrocytes (4) as well as other cell types, including neural cells (5, 6), and they play important roles in the embryonal development (3). More than a dozen proteins belong to the BMP family, including BMP-2 to -6, osteogenic protein (OP)-1 (also termed BMP-7), OP-2 (BMP-8), and growth/differentiation factors 5 to 7.

BMPs belong to the transforming growth factor β (TGF-β) superfamily, which includes TGF-βs, activins/inhibins, Müllerian inhibiting substance (MIS), and glial cell line-derived neurotrophic factor (GDNF) (7). TGF-βs and activins transduce their signals through the formation of heteromeric complexes of two different types of serine(threonine) kinase receptors—i.e., type I receptors of about 50–55 kDa and type II receptors of about 70–80 kDa (8, 9). Type II receptors bind ligands in the absence of type I receptors, but they require their respective type I receptors for signaling, whereas type I receptors require their respective type II receptors for ligand binding.

Six different type I serine(threonine) kinase receptors have been identified in mammals (10–19), including a TGF-β type I receptor (TβR-I) (13, 15, 16), two activin type I receptors (ActR-I and ActR-IB) (12, 14, 16, 17), and two BMP type I receptors (BMPR-IA and BMPR-IB) (18, 19). BMP-4 binds to BMPR-IA and BMPR-IB efficiently (18, 19) in the presence of DAF-4, a BMP type II receptor in *Caenorhabditis elegans* (20), whereas OP-1/BMP-7 binds to BMPR-IB and, less efficiently, to BMPR-IA. Moreover, OP-1/BMP-7 can bind ActR-I in the presence of DAF-4 (18). BMP-4 ventralizes *Xenopus* embryos when it is overexpressed (21, 22). Truncated forms of BMPR-IA block the effects of BMPs and convert ventral mesoderm to dorsal mesoderm (23, 24).

Type II receptors for activin (ActR-II and ActR-IIB) and for TGF-β (TβR-II) have been identified in mammals (25–28), but BMP type II receptors have thus far been identified only in *C. elegans* (20). We report here a human type II serine(threonine) kinase receptor (BMPR-II)** that binds OP-1/BMP-7 and BMP-4 and is distantly related to DAF-4. Moreover, we show that BMPR-II transduces a signal in the presence of type I receptors after stimulation by OP-1/BMP-7.

MATERIALS AND METHODS

cDNA Cloning. Poly(A)$^+$ RNA was isolated from adult rat substantia nigra tissue, and first-strand cDNA was synthesized. Degenerate polymerase chain reaction (PCR) primers were designed based on the kinase domain regions VIII and XI (29) of serine(threonine) kinase receptors, and inosines (I) were used to reduce degeneracy. The sense primer sequence was 5′-GGGAG GGAAT TC(AC)G(GATC) TA(TC)AT GGC-(GATC)C C(GATC)CA (GA) GTI(TC)T-3′ and the antisense primer sequence was 5′-GGGAG GGAAT TCTC(GATC) G(GC)(GA)T(TC) ITG(GA)T (AC)CCA(GA) CA(TC)TC-3′. PCR was performed using a PCR Gem hot start protocol (Perkin–Elmer/Cetus). The resulting products were ligated into pBluescript II SK$^-$ (Stratagene) and the sequences were determined using a Dye-Deoxy Terminator cycle sequencing kit (Applied Biosystems). Analysis of the PCR products revealed a clone with a new sequence, denoted U2. The rat U2 PCR fragment was labeled with [α-^{32}P]dCTP by PCR and was hybridized to a human substantia nigra cDNA library in λgt10 (Clontech) at reduced stringency. Five strongly hybridizing plaques were picked; the largest cDNA with a 4-kb insert was subcloned into pBluescript II SK and sequenced by primer walking using Dye-Deoxy Terminator cycle sequencing (Applied Biosystems).

Northern Blot Analysis. Poly(A)$^+$ RNAs from adult and fetal human tissues (Clontech) were electrophoresed on agarose/formaldehyde gels and transferred to Hybond N membranes (Amersham). A portion of the human BMPR-II cDNA from 93 bp upstream of the translation initiation ATG codon to bp 2518 was amplified by PCR and ^{32}P-labeled by using the Quick Prime kit (Pharmacia). Hybridization conditions were 0.5 M sodium phosphate (pH 7.4), 7% SDS, 1 mM EDTA, and 200 μg of yeast tRNA per ml at 68°C overnight, followed by

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Abbreviations: ActR, activin receptor; BMP, bone morphogenic protein; BMPR, BMP receptor; BS^3, bis(sulfosuccinimidyl) suberate; GDNF, glial cell line-derived neurotrophic factor; MIS, Müllerian inhibiting substance; OP, osteogenic protein; TGF-β, transforming growth factor β; TβR, TGF-β receptor.

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**The sequence reported in this paper has been deposited in the GenBank data base (accession no. Z48923).

high temperature washes at 68°C and autoradiography. A Northern blot filter with mRNAs from different adult human tissues (Clontech) was hybridized with a *Pst* I restriction fragment of the BMPR-II cDNA (nucleotides 18–807) radiolabeled by the Megaprime DNA labeling system, washed, and subjected to autoradiography, as described (11).

Cell Culture. COS-1 cells were obtained from the American Type Culture Collection. R mutant mink lung epithelial (Mv1Lu) cells (30) and U-1240 MG human glioblastoma cells (31) were obtained from M. Laiho (University of Helsinki) and J. Massagué (Memorial Sloan–Kettering Cancer Center) and Bengt Westermark (University of Uppsala), respectively. Cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 100 units of penicillin per ml, and 50 μg of streptomycin per ml.

Transient Transfection. Transient expression plasmids encoding type I receptors and *daf-4* (obtained from D. L. Riddle, University of Missouri) were previously described (16, 18). BMPR-II cDNA was subcloned into pcDNA3 expression vector (Invitrogen). These plasmids and a p3TP-Lux promoter-reporter construct (obtained from J. Massagué) were transfected into COS-1 cells or R mutant Mv1Lu cells by a calcium phosphate precipitation method with an MBS mammalian transfection kit (Stratagene) (16, 18).

Recombinant Proteins and Radiolabeling. Recombinant human OP-1/BMP-7 (32), BMP-4 (33), TGF-β1, and activin A were obtained from T. K. Sampath (Creative BioMolecules, Hopkinton, MA), A. H. Reddi (Johns Hopkins University), H. Ohashi (Kirin Brewery, Maebashi, Japan), and Y. Eto (Ajinomoto Co., Kawasaki, Japan), respectively. Recombinant human GDNF was prepared as described (34). OP-1/BMP-7 and BMP-4 were iodinated using the chloramine-T method as described (18). ^{125}I-labeled OP-1/BMP-7 (^{125}I-OP-1/BMP-7) was observed as multiple components of 16–19 kDa under reducing condition. ^{125}I-BMP-4 migrated slightly slower than ^{125}I-OP-1/BMP-7 in SDS gel electrophoresis.

Preparation of Polyclonal Antibodies. Antisera to type I receptors were made against synthetic peptides corresponding to the intracellular juxtamembrane parts of type I receptors (16). Antisera against BMPR-II (SMN and NRR) were generated against peptides corresponding to amino acid residues 185–202 and 534–556, respectively (see Fig. 1*A*). An antiserum to DAF-4 was raised against a peptide corresponding to amino acid residues 273–294 (20). Peptides were coupled to keyhole limpet hemocyanin (35) and injected into rabbits as described (36). SMN and NRR antisera recognized BMPR-II equally well; therefore, a mixture of SMN and NRR (1:1) was used, unless otherwise specified.

Binding, Affinity Cross-Linking, and Immunoprecipitation. Cells were incubated on ice for 2–3 hr with 0.2–0.5 nM of ^{125}I-labeled ligands in the presence or absence of unlabeled ligands in the binding buffer (16, 18). After incubation, the cells were washed and cross-linking was done using 1 mM bis(sulfosuccinimidyl) suberate (BS^3) (Pierce) for 15 min on ice. The cells were washed, scraped off the plates, centrifuged, and resuspended in solubilization buffer, followed by incubation for 20 min on ice. Immunoprecipitation of the cross-linked materials was performed as described (18). The immune complexes were eluted by boiling 3 min in SDS sample buffer with 10 mM dithiothreitol and subjected to SDS gel electrophoresis, followed by autoradiography or analysis using a Bio-Imaging analyzer (BAS 2000) (Fuji).

Transcriptional Response Assay. R mutant Mv1Lu cells were cotransfected with p3TP-Lux promoter-reporter construct (12, 37) with plasmids containing type II or type I receptor cDNAs as described above. Cells were washed with phosphate-buffered saline on the following day. The cells were starved in Dulbecco's modified Eagle's medium containing 0.1% fetal bovine serum, 100 units of penicillin per ml, and 50 μg of streptomycin per ml for 6 hr and then exposed to various concentrations of OP-1/BMP-7 for 24 hr. Luciferase activity in the cell lysate was measured using the luciferase assay system (Promega) according to the manufacturer's protocol and a luminometer (model 1250; LKB).

RESULTS AND DISCUSSION

Cloning of a Serine(Threonine) Kinase Receptor. To obtain receptors for neurotrophic factors of the TGF-β superfamily, PCR was performed on adult rat substantia nigra cDNA using primers from kinase domains of serine(threonine) kinase receptors. Analysis of the clones obtained by this strategy revealed a clone with a new sequence, termed U2. A full-length cDNA was isolated from a human substantia nigra cDNA library by plaque hybridization using the rat U2 as a probe. The largest cDNA with a 4-kb insert was found to encode a protein (BMPR-II) of 1038 amino acid residues comprising an N-terminal hydrophobic leader sequence, followed by an extracellular domain, a single transmembrane domain, and an intracellular region with a serine(threonine) kinase domain (Fig. 1*A*). BMPR-II lacks a glycine- and serine-rich sequence in the juxtamembrane domain, which is characteristic of type I receptors (7–9), and is considerably larger (about 115 kDa) than previously described mammalian serine(threonine) kinase receptors due to

A

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MTSSLQRPWRVPWLPWTILLVSTAAASQNQERLCAFKDPYQQDLGIGESR      50
ISHENGTILCSKGSTCYGLWEKSKGDINLVKQGCWSHIGDPQECHYEECV     100
VTTTPPSIQNGTYRFCCCSTDLCNVNFTENFPPPDTTPLSPPHSFNRDET     150
ITTALASVSVLAVLIVALCFGYRMLTGDRKQGLHSMNMMEAAASEPSLDL     200
DNLKLLELIGRGRYGAVYKGSLDERPVAVKVFSFANRQNFINEKNIYRVP     250
LMEHDNIARFIVGDERVTADGRMEYLLVMEYYPNGSLCKYLSLHTSDWVS     300
SCRLAHSVTRGLAYLHTELPRGDHYKPAISHRDLNSRNVLVKNDGTCVIS     350
DFGLSMRLTGNRLVRPGEEDNAAISEVGTIRYMAPEVLEGAVNLRDCESA     400
LKQVDMYALGLIYWEIFMRCTDLFPGESVPEYQMAFQTEVGNHPTFEDMQ     450
VLVSREKQRPKFPEAWKENSLAVRSLKETIEDCWDQDAEARLTAQCAEER     500
MAELMMIWERNKSVSPTVNPMSTAMQNERNLSHNRRVPKIGPYPDYSSSS     550
YIEDSIHHTDSIVKNISSEHSMSSTPLTIGEKNRNSINYERQQAQARIPS     600
PETSVTSLSTNTTTTNTTGLTPSTGMTTISEMPYPDETNLHTTNVAQSIG     650
PTPVCLQLTEEDLETNKLDPKEVDKNLKESSDENLMEHSLKQFSGPDPLS     700
STSSSLLYPLIKLAVEATGQQDFTQTANGQACLIPDVLPTQIYPLPKQQN     750
LPKRPTSLPLNTKNSTKEPRLKFGSKHKSNLKQVETGVAKMNTINAAEPH     800
VVTVTMNGVAGRNHSVNSHAATTQYANRTVLSGQTTNIVTHRAQEMLQNQ     850
FIGEDTRLNINSSPDEHEPLLRREQQAGHDEGVLDRLVDRRERPLEGGRT     900
NSNNNNSNPCSEQDVLAQGVPSTAADPGPSKPRRAQRPNSLDLSATNVLD     950
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B

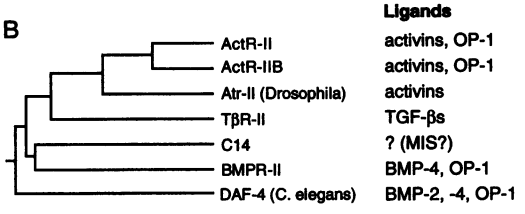

FIG. 1. Cloning of BMPR-II. (*A*) Deduced amino acid sequence of the human BMPR-II cDNA. The putative hydrophobic leader sequence and transmembrane domain are double overlined. Cysteine residues in the extracellular domain are boxed, and potential N-glycosylation sites are overlined by thick lines. The borders of the kinase domain are shown by arrows. Amino acid sequences used for preparing antisera are underlined (thin lines). (*B*) Phylogenetic tree based on the amino acid sequence similarities between the kinase domains of type II serine(threonine) kinase receptors. ActR-II (25), ActR-IIB (26), TβR-II (28), C14 (38, 39), and BMPR-II are from mammals, Atr-II (40) is from *Drosophila*, and DAF-4 (20) is from *C. elegans*. ActR-II and ActR-IIB bind activins and OP-1/BMP-7 (H.Y. and P.t.D., unpublished data).

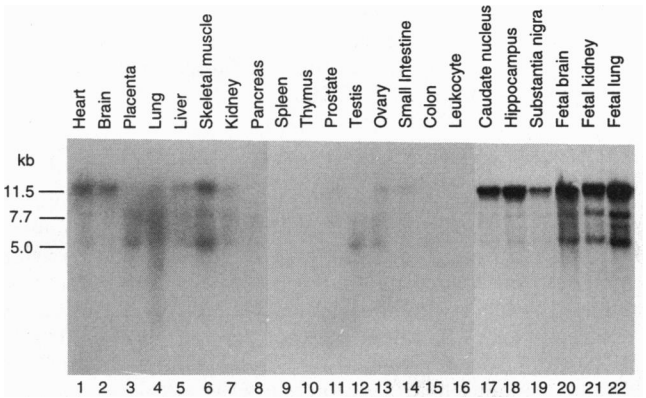

FIG. 2. Expression of BMPR-II mRNA in various human tissues. Blots of mRNAs from different human tissues were hybridized using human BMPR-II probes (corresponding to nucleotides 18–807 for lanes 1–16 and nucleotides −93–2518 for lanes 17–22). All tissues are adult except where a fetal source is indicated.

the presence of a long C-terminal tail rich in serine and threonine residues (22%).

Comparison of the amino acid sequence of the kinase domain of BMPR-II revealed that it is likely to have specificity for serine(threonine) residues, but it is only distantly related to the other serine(threonine) kinase receptors, including DAF-4 (Fig. 1*B*). There are 10 cysteine residues in the extracellular domain, which could be well aligned with those of the other serine(threonine) kinase receptors; however, the amino acid sequence identity in the extracellular domain of BMPR-II is <28% compared to other serine(threonine) kinase receptors. No sequence similarity between the long C-terminal tail and other known sequences was found.

Expression of BMPR-II mRNA. Hybridization of BMPR-II probes to human mRNAs from different adult and fetal tissues indicated that BMPR-II mRNA is widely distributed in different tissues, including heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, prostate, testis, ovary, and small intestine. Representative Northern blots are shown in Fig. 2. Major transcripts of 11.5 kb, 7.7 kb, and 5.0 kb were present in all tissues and a minor transcript of 6.6 kb was seen in some tissues. Weak or no hybridization signals were detected in spleen, thymus, colon, and leukocytes. Expression levels in the examined fetal tissues were similar to those in adult tissues (Fig. 2 and data not shown).

Binding of OP-1/BMP-7 and BMP-4 to BMPR-II. To identify ligands for BMPR-II, binding studies using ^{125}I-labeled members of the TGF-β superfamily were performed. cDNAs for BMPR-II and various type I receptors were transfected singly or together into COS-1 cells; cells were then incubated with various ^{125}I-labeled ligands, washed, and subjected to cross-linking with a homobifunctional cross-linker. Samples were then analyzed by SDS gel electrophoresis after immunoprecipitation using antisera to BMPR-II or type I receptors. BMPR-II alone bound ^{125}I-OP-1/BMP-7 and very weakly bound BMP-4 (Fig. 3 *A* and *B*). However, BMPR-II alone or cotransfected with the six different mammalian type I receptors did not bind TGF-β1, activin A, or GDNF. Affinity cross-linking studies using ^{125}I-OP-1/BMP-7 revealed that BMPR-II bound OP-1/BMP-7 and formed a cross-linked complex of 130 kDa and a less abundant complex of 145 kDa (Fig. 3*A*). When the cDNA for BMPR-II was cotransfected with cDNAs for ActR-I or BMPR-IB, which are known to bind ^{125}I-OP-1/BMP-7 in the presence of DAF-4 (18), the abundance of the 130-kDa complex was increased and complexes of 190, 90–95, and 82–74 kDa could also be seen. The 82- to 74-kDa components represent type I receptors (ActR-I or BMPR-IB) (18). The other components may represent oligomer(s) of BMPR-II and type I receptors or receptors cross-linked to two rather than one of the subunits in the OP-1/BMP-7 dimer. Similar multiple bands have also been identified when COS-1 cells were transfected with *daf-4* cDNA together with the corresponding type I receptors (ref. 18; see

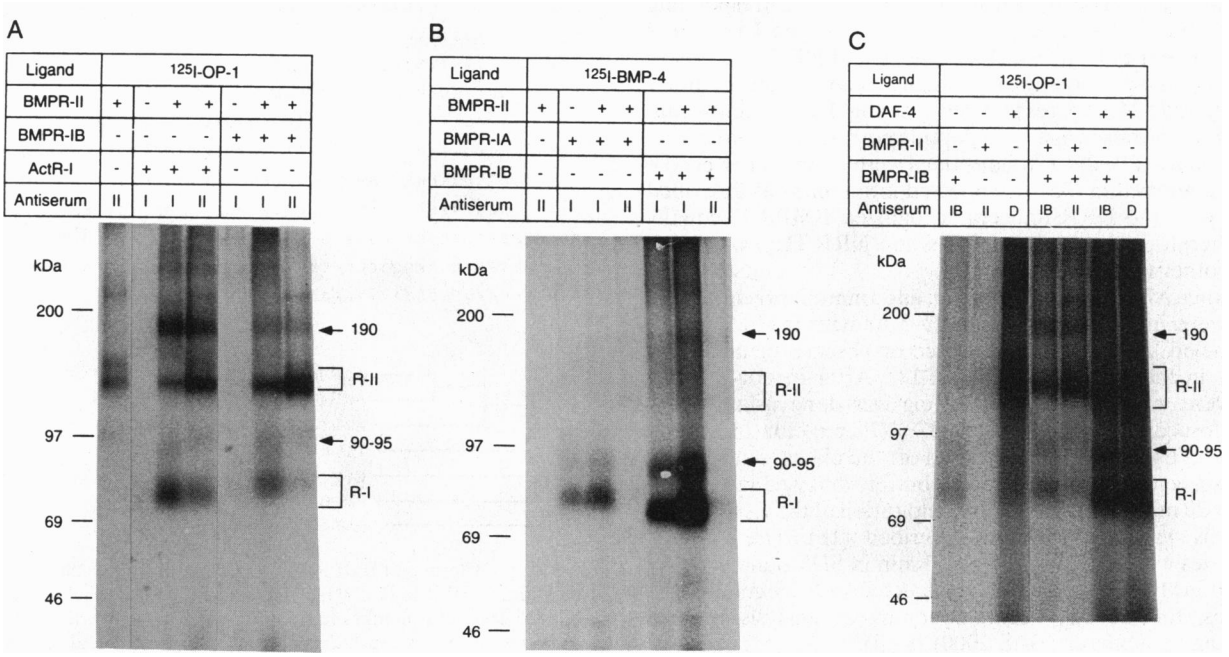

FIG. 3. Binding of OP-1/BMP-7 and BMP-4 to BMPR-II. (*A* and *B*) Binding of OP-1/BMP-7 and BMP-4 to BMPR-II in the presence of type I receptors. COS-1 cells were transfected with cDNAs for BMPR-II, BMPR-IB, ActR-I, or BMPR-IA, alone or in combination, and affinity labeled with ^{125}I-OP-1/BMP-7 (*A*) or ^{125}I-BMP-4 (*B*). Receptors were cross-linked with BS^3, immunoprecipitated with antisera to BMPR-II (II) or corresponding type I receptors (I), and analyzed by SDS gel electrophoresis followed by autoradiography. (*C*) Comparison of the binding of ^{125}I-OP-1/BMP-7 to BMPR-II and DAF-4. COS-1 cells were transfected with cDNAs for *daf-4*, BMPR-II, or BMPR-IB, alone or in combination, affinity labeled by ^{125}I-OP-1/BMP-7, and cross-linked by BS^3. Immunoprecipitation was done using antisera to BMPR-IB (IB), BMPR-II (II), or DAF-4 (D); samples were analyzed by SDS gel electrophoresis followed by analysis using a Bio-Imaging analyzer. Positions of type II receptors (R-II), type I receptors (R-I), and other cross-linked complexes are indicated on the right.

also Fig. 3*C*). The fact that type II receptor complexes were coimmunoprecipitated with antisera against the type I receptors and vice versa indicates that ligand binding induces a complex of type I and type II receptors. The binding of ^{125}I-OP-1/BMP-7 was competitively inhibited by excess amounts of unlabeled OP-1/BMP-7. The weak binding of ^{125}I-OP-1/BMP-7 to BMPR-IA was also up-regulated upon cotransfection with BMPR-II. Other mammalian type I receptors (TβR-I, ActR-IB, and activin receptor-like kinase 1) did not bind OP-1/BMP-7 in the presence of BMPR-II (data not shown).

The binding of ^{125}I-BMP-4 to BMPR-II was less efficient than that of ^{125}I-OP-1/BMP-7. When the cDNA for BMPR-II was cotransfected with BMPR-IA or BMPR-IB, and cells were subjected to binding and cross-linking using ^{125}I-BMP-4, complexes of 130, 90–95, and 82–74 kDa were immunoprecipitated by antisera to type I receptors or BMPR-II (Fig. 3*B*), but only low amounts of receptor complexes were immunoprecipitated by the BMPR-II antisera compared to the type I receptor antisera. The coimmunoprecipitation of BMPR-IA or BMPR-IB with BMPR-II indicates that both type I receptors can form heteromeric complexes with BMPR-II after ligand binding of BMP-4. We have previously shown that ^{125}I-BMP-4 binds to BMPR-IA and BMPR-IB in the presence of DAF-4 (18). In contrast, the presence of BMPR-II increased the binding of BMP-4 to BMPR-IB but only weakly to BMPR-IA.

The binding of ^{125}I-OP-1/BMP-7 to BMPR-II was compared with the binding to the *C. elegans* BMP type II receptor DAF-4 in the presence of BMPR-IB. When transfected singly, ^{125}I-OP-1/BMP-7 bound to DAF-4 and to BMPR-II less efficiently (Fig. 3*C*). In the presence of BMPR-IB, the binding to BMPR-II increased, but not the binding to DAF-4. Cross-linked complexes of BMPR-II migrated slightly slower than those of DAF-4, consistent with the larger molecular mass of BMPR-II than that of DAF-4.

Expression of BMPR-II in Cultured Cell Lines. We have previously shown that ^{125}I-labeled BMPs bind BMPR-IA, BMPR-IB, or ActR-I in certain cultured cell lines, including Mv1Lu cells and U-1240 MG glioblastoma cells (18). The binding of ^{125}I-OP-1/BMP-7 to receptors endogenously expressed in cultured cells was studied using an antiserum to BMPR-II (NRR). Immunoprecipitation of the cross-linked complexes revealed that type II receptor complexes of 130 kDa, as well as other components that may represent coimmunoprecipitated type I receptors and oligomer(s) of type I and/or type II receptors, could be observed in U-1240 MG glioblastoma cells, R mutant Mv1Lu cells (Fig. 4*A*), and wild-type Mv1Lu cells (data not shown). A component of 165 kDa was seen in these cells; whether this component represents an oligomer of BMPR-II and/or type I receptor or an alternatively spliced variant of BMPR-II remains to be elucidated. Similar results were obtained using another BMPR-II antiserum (SMN). When transfected into COS-1 cells, BMPR-IB bound ^{125}I-OP-1/BMP-7 and ^{125}I-BMP-4 in the absence of a cotransfected type II receptor (18). To test whether this may be due, at least in part, to the presence of an endogenous BMP type II receptor in COS-1 cells, cDNA for BMPR-IB was transfected into COS-1 cells, and cross-linked complexes were immunoprecipitated by antiserum to BMPR-II. As shown in Fig. 4*B*, a cross-linked complex of 130 kDa could be immunoprecipitated by the BMPR-II antiserum in these cells, supporting the notion that COS-1 cells endogenously express BMPR-II. We have confirmed by Northern blot analysis that COS-1 cells express BMPR-II mRNA (data not shown).

COS cells singly transfected with BMPR-IB appear to have higher affinity for BMP-4 than OP-1 (Fig. 3). It is thus possible that COS cells in addition express another BMP type II receptor that binds more avidly to BMP-4 than OP-1.

Signaling Activity of BMPR-II. We next investigated the signaling activity of BMPR-II using a p3TP-Lux promoter-

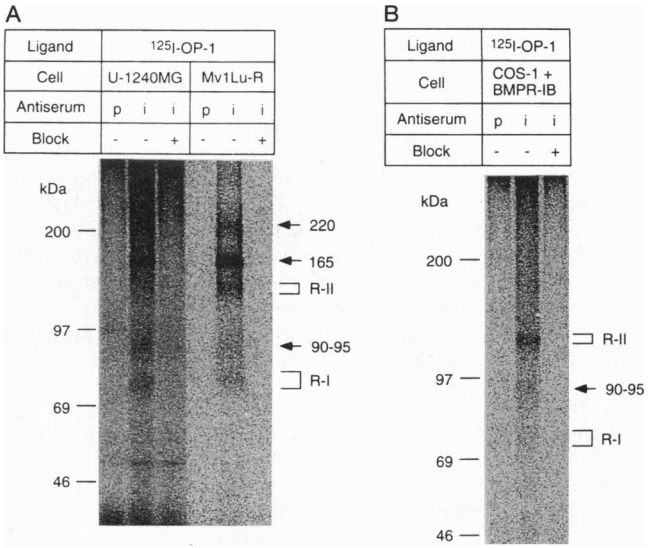

FIG. 4. Identification of endogenous BMPR-II in different cell lines. (*A*) Binding of ^{125}I-OP-1/BMP-7 to U-1240 MG glioblastoma cells and R mutant Mv1Lu cells (Mv1Lu-R). (*B*) Binding of ^{125}I-OP-1/BMP-7 to COS-1 cells transfected with BMPR-IB cDNA. After cross-linking, samples were immunoprecipitated by the immune (i) or preimmune (p) serum to BMPR-II (NRR) in the presence or absence of the peptide used for immunization (Block); samples were analyzed by SDS gel electrophoresis, followed by analysis using a Bio-Imaging analyzer (*A*) or by autoradiography (*B*). Positions of BMPR-II (R-II), type I receptors (R-I), and other cross-linked complexes are indicated on the right.

reporter construct (12, 37). R mutant Mv1Lu cells, which are highly transfectable and suitable for the p3TP-Lux assay, were used. Although the R mutant cells express endogenous receptors for activins (16) and BMPs (Fig. 4*A*), superinduction of a transcriptional signal could be detected after ligand stimulation of cells cotransfected with cDNAs for ActR-IIs and ActR-I in this assay system (ref. 12; H.Y. and P.t.D., unpublished data). When R mutant cells were transfected with a single receptor cDNA (BMPR-II, ActR-I, or BMPR-IB) together with the p3TP-Lux plasmid, transcription was not activated after addition of OP-1/BMP-7 (Fig. 5). When p3TP-Lux was cotransfected with BMPR-II and a type I receptor (ActR-I or BMPR-IB) into R mutant cells, transcriptional activation was observed after stimulation by OP-1/BMP-7

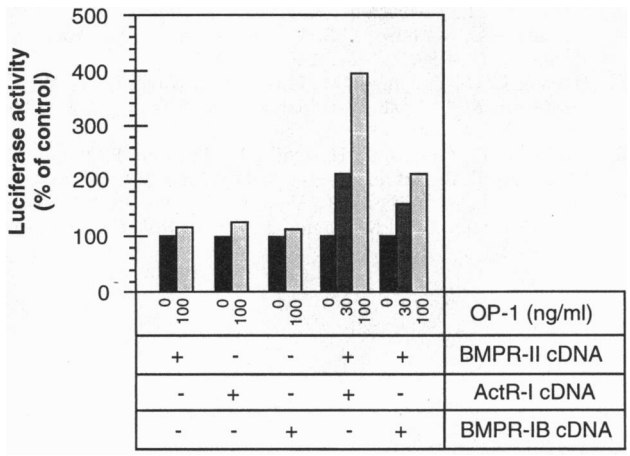

FIG. 5. Signal transduction by BMPR-II. cDNAs for BMPR-II, ActR-I, and BMPR-IB were cotransfected with p3TP-Lux into R mutant of Mv1Lu cells. The transcriptional response after stimulation by various concentrations of OP-1/BMP-7 was determined by a luciferase activity.

(Fig. 5). These results suggest that BMPR-II forms heteromeric complexes with the corresponding type I receptors in the presence of OP-1/BMP-7 and transduces signals.

CONCLUSION

We report here the cloning of a human type II receptor for members of the BMP family. In contrast to the type II receptors for TGF-β and activin, efficient ligand binding to BMPR-II is dependent on the presence of appropriate type I receptors. However, the general concept for signal transduction by TGF-β and activin appears to apply also for BMPs; heteromeric receptor complexes consisting of type I and type II receptors are needed for signaling.

Note. The cloning of a sequence denoted T-ALK was recently reported (41). The sequence is essentially identical to the sequence of BMPR-II.

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