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Human Type II Receptor for Bone Morphogenic Proteins (BMPs): Extension of the Two-Kinase Receptor Model to the BMPs

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Bone morphogenic proteins (BMPs) are universal regulators of animal development. We report the identification and cloning of the BMP type II receptor (BMPR-II), a missing component of this receptor system in vertebrates. BMPR-II is a transmembrane serine/threonine kinase that binds BMP-2 and BMP-7 in association with multiple type I receptors, including BMPR-IA/Brk1, BMPR-IB, and ActR-I, which is also an activin type I receptor. Cloning of BMPR-II resulted from a strong interaction of its cytoplasmic domain with diverse transforming growth factor beta family type I receptor cytoplasmic domains in a yeast two-hybrid system. In mammalian cells, however, the interaction of BMPR-II is restricted to BMP type I receptors and is ligand dependent. BMPR-II binds BMP-2 and -7 on its own, but binding is enhanced by coexpression of type I BMP receptors. BMP-2 and BMP-7 can induce a transcriptional response when added to cells coexpressing ActR-I and BMPR-II but not to cells expressing either receptor alone. The kinase activity of both receptors is essential for signaling. Thus, despite their ability to bind to type I and II receptors separately, BMPs appear to require the cooperation of these two receptors for optimal binding and for signal transduction. The combinatorial nature of these receptors and their capacity to crosstalk with the activin receptor system may underlie the multifunctional nature of their ligands.

The biology of the bone morphogenetic proteins (BMPs) exemplifies the concept that diverse developmental events in phylogenetically distant organisms are regulated by discrete groups of highly conserved gene products. BMPs were first identified in mammals as osteoinductive cytokines from the bone matrix (50), and their genetic loss can cause marked skeletal defects (25). Remarkably, their *Drosophila* homolog, the decapentaplegic gene product (Dpp), is a crucial dorsalizing morphogen and a promoter of eye, gut, and appendage formation (15, 20, 38). The multifunctional nature of BMPs is also apparent in vertebrates, in which they are implicated in many processes besides bone formation (19, 24, 25, 31).

As general regulators of animal development, BMPs are thought to act through a broadly conserved mechanism, but this point has remained unproven because of difficulties in identifying BMP receptors. Clues about the nature of these receptors come from the fact that BMPs are members of the transforming growth factor beta (TGF-β) family (25, 33). The TGF-βs and the related activins initiate their actions by contacting two types of transmembrane serine/threonine kinases, known as receptors I and II (33, 36). TGF-β and activin bind first to their specific type II receptor (TβR-II), and the ligand in this complex is then recognized by type I receptors, which cannot bind ligand otherwise. Once recruited, type I receptors become phosphorylated by the associated type II receptor and propagate the signal (4, 52). Phosphorylation occurs in the GS domain, a motif immediately upstream of the kinase domain and conserved in all known type I receptors (52). Accordingly, the type II receptor acts, despite its name, as the primary receptor, and the type I receptor acts as its substrate and downstream signaling component.

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The type I receptors for the TGF-β family are closely related kinases (25, 33, 36). Type I receptors for TGF-β and activin require the presence of a type II receptor in order to bind ligand. However, members of this family from mammals, *Xenopus laevis*, and *Drosophila melanogaster* have been recently shown to be capable of binding BMP or Dpp on their own (7, 17, 27, 39, 43, 45). This property raised doubts as to whether formation of heteromeric kinase receptor complexes is required for signal transduction by the BMPs. Since BMPs are the most phylogenetically conserved members of the TGF-β family, it could be that their receptor scheme is simpler than those of TGF-βs and activins, factors that are found only in vertebrates (25, 33). A member of the type II receptor family involved in dauer larva formation in *Caenorhabditis elegans*, Daf-4, can bind human BMPs (14) and augment binding to mammalian and *Drosophila* BMP type I receptors (BMPR-I) (7, 27, 39, 45). However, the natural ligand and signaling properties of Daf-4 are unknown. Since no BMP type II receptor has been described to date, the involvement of heteromeric kinase receptor complexes in BMP signaling has remained an open question.

Unlike type I receptors, the type II receptors for TGF-β and related factors are structurally quite divergent (3), and their identification has often relied on their ability to bind ligand in expression cloning approaches (29, 34). However, we recently observed that the cytoplasmic domains of the TGF-β type I and type II receptors can interact in a yeast two-hybrid system (46), suggesting that novel members of this receptor family could be cloned by using this system. Here we describe the cloning of a human BMP type II receptor (BMPR-II) using the yeast two-hybrid system and demonstrate its ability to bind ligand cooperatively with BMPR-I, forming heteromeric complexes required for generation of a cellular response. The ligand-receptor interactions reported here are remarkably similar to those recently observed with the Dpp receptor system from *D. melanogaster* (28, 40).

MATERIALS AND METHODS

Yeast two-hybrid screening and cloning of full-length BMPR-II cDNA. The yeast two-hybrid system used has been described (18, 53). The entire cytoplasmic domain of human TβR-I (amino acids 148 to 503) (16) was fused in frame with the LexA DNA-binding domain in the vector pEG202. Introduction of this bait (pEG202-TβR-I) into the EGY48 strain along with the reporter pSH18-34 gave very low basal reporter transcription. Screening of 10^7 clones of an oligo(dT)-primed library from human HeLa cells was done as previously described (18, 53). Fourteen identical clones encoding the BMPR-II kinase domain were isolated. To isolate a full-length BMPR-II cDNA, a λgt11 human kidney library was screened with the kinase domain as a probe.

For interaction assays with *Saccharomyces cerevisiae*, the cytoplasmic domains of ActR-I (amino acids 147 to 509) (1), ActR-IB (amino acids 150 to 505) (8), and TSR-I (amino acids 144 to 503) (1) were fused in frame to the LexA DNA-binding domain in the vector pEG202. Yeast growth and transformation were carried out by standard protocols.

Mammalian expression vectors and transfections. A full-length BMPR-II cDNA, including 28 bp of 5′ untranslated region (UTR), was subcloned into pCMV5. The BMPR-II^{His} and BMPR-II^{Flag} constructs, encoding a hexahistidine sequence and the Flag epitope sequence (21), respectively, at the C terminus, were generated with annealed oligonucleotides. Lysine-to-arginine mutations at the ATP binding site of ActR-I (K235R) and BMPR-II (K230R) were generated by PCR with the corresponding viral hemagglutinin (HA)- and histidine-tagged versions as templates. Generation of TβR-II^{His}, TβR-I^{HA}, TSR-I^{HA}, ActR-I^{HA}, and p3TP-Lux vectors in pCMV5 has been described previously (1, 8). The BMPR-IA(ALK3) cDNA in pcDNA1 and BMPR-IB(ALK6) cDNA in pSV7d were kindly provided by K. Miyazono (45). Cell lines COS-1, Mv1Lu, R-1B/L-17, and DR-26 were cultured and transiently transfected with the indicated vectors as previously described (1, 8) and assayed at 48 to 72 h posttransfection.

Receptor affinity labeling assays. TGF-β1 from porcine platelets (R&D Systems), activin A (Genentech), BMP-2 (Genetics Institute), and OP-1/BMP-7 (Creative Biomolecules) were labeled with ^{125}I (10). Cell monolayers were incubated for 3 h at 4°C with 250 pM ^{125}I-TGF-β1 or ^{125}I-activin A or 500 pM ^{125}I-BMP-2 or ^{125}I-BMP-7. Receptors were cross-linked to bound ligands with disuccinimidyl suberate (Pierce Chemical Co.) and solubilized in the presence of Triton X-100 (32). Cell extracts were clarified by centrifugation and subjected to purification and/or to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography.

Purification of receptor complexes. Affinity-labeled cells or [^{35}S]methionine-labeled cells (2 h in methionine-free medium containing 50 μCi of Trans ^{35}S-Label [ICN] per ml) were lysed for 20 min in lysis buffer (50 mM Tris-Cl [pH 7.4], 150 mM NaCl, 0.5% [vol/vol] Triton X-100, protease inhibitors). For isolation of receptor complexes, cell extracts were clarified by centrifugation, brought to 20 mM imidazole, incubated with Ni^{2+}-nitrilotriacetic acid (NTA)-agarose (Qiagen) for 1 h at 4°C, and rinsed briefly five times with 20 mM imidazole in lysis buffer. Ni^{2+}-NTA-agarose-bound receptors were eluted with 250 mM imidazole in lysis buffer. Eluates were diluted fivefold with lysis buffer and precipitated with HA antibody (12CA5; Babco) or M2 Flag antibody (IBI), followed by binding to protein G-Sepharose (Pharmacia). Immunoprecipitates were washed three times with lysis buffer and three times with RIPA buffer (10 mM Tris-HCl, 1% sodium deoxycholate, 0.5% Triton X-100, 0.1% SDS [pH 7.5]) and subjected to SDS-PAGE and autoradiography.

Luciferase assay. One day after transfections, cells were seeded into 12-well plates and incubated in medium containing 0.2% fetal bovine serum for 14 to 16 h with addition of 250 pM TGF-β1, 250 pM activin A, 2 nM BMP-2, or 2 nM BMP-7 as previously described (1, 8). The cells were harvested, and luciferase activity in cell lysates was determined with a luciferase assay system (Promega) in a Berthold Lumat LB 9501 luminometer.

Nucleotide sequence accession number. The human BMPR-II sequence has been deposited in the EMBL data bank and assigned EMBL accession number U25110.

RESULTS

Cloning of BMPR-II as a type I receptor-interacting protein in yeast cells. In order to identify proteins that interact with type I receptors for the TGF-β family, we fused the cytoplasmic domain of the human TGF-β type I receptor TβR-I (16) to the LexA DNA-binding domain for use as bait in a yeast two-hybrid system (18, 53) to screen a human HeLa cell cDNA library. Of 10^7 clones screened, 21 interacted strongly and specifically with TβR-I. Sequence analysis revealed that seven of these clones encoded the FK506 binding protein FKBP12 (41), previously identified as a TβR-I-interacting protein in yeast cells (47) and in mammalian cells (30). The other 14 clones encoded a canonical serine/threonine kinase domain

A

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MTSSLQRPWRVPWLPWTILLVSTAAASQNQERLCAFKDPYQQDLGIGESR   50
ISHENGTILCSKGSTCYGLWEKSKGDINLVKQGCWSHIGDPQECHYEECV  100
VTTTPPSIQNGTYRFCCCSTDLCNVNFTENFPPPDTTPLSPPHSFNRDET  150
IIIALASVSVLAVLIVALCFGYRMLTGDRKQGLHSMNMMEAAASEPSLDL  200
DNLKLLELIGRGRYGAVYKGSLDERPVAVKVFSFANRQNFINEKNIYRVP  250
LMEHDNIARFIVGDERVTADGRMEYLLVMEYYPNGSLCKYLSLHTSDWVS  300
SCRLAHSVTRGLAYLHTELPRGDHYKPAISHRDLNSRNVLVKNDGTCVIS  350
DFGLSMRLTGNRLVRPGEEDNAAISEVGTIRYMAPEVLEGAVNLRDCESA  400
LKQVDMYALGLIYWEIFMRCTDLFPGESVPEYQMAFQTEVGNHPTFEDMQ  450
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B

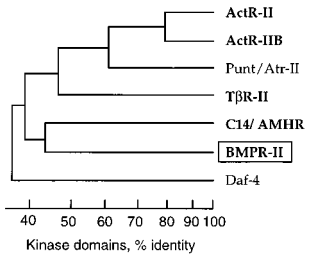

FIG. 1. Human BMPR-II amino acid sequence and relationship to type II receptor kinases. (A) The extracellular cysteines, putative transmembrane region (underlined), and the limits of the kinase domain (arrow brackets) are indicated. (B) Relationship dendrogram generated with the Geneworks program and based on the deduced amino acid sequences of the mammalian receptors (shown in boldface type) ActR-II (34), ActR-IIB (2), TβR-II (29), and C14/AMHR (5, 13); the *Drosophila* receptor Punt/Atr-II (12); and the *C. elegans* receptor Daf-4 (14).

(Fig. 1A), preceded by seven amino acids and followed by a short C-terminal extension and 3′ untranslated region but lacking a start codon. A search of current sequence databases indicated that this kinase domain resembles most closely those of type II receptors for the TGF-β family (Fig. 1B). From the properties described below, we refer to this novel kinase as BMPR-II.

Sixty additional positive clones that interacted less strongly with the TβR-I construct included multiple FKBP12 isolates but not BMPR-II, suggesting that this kinase was the strongest TβR-I-interacting protein in the library. To determine whether BMPR-II could interact with the cytoplasmic domains of other type I receptors, we tested it in the yeast two-hybrid system against LexA DNA-binding domain baits containing the cytoplasmic domains of the activin/BMP type I receptor ActR-I (1), the activin type I receptor ActR-IB (8, 44), and the activin/TGF-β type I receptor TSR-I (1). BMPR-II interacted strongly with all type I receptor constructs mentioned above but not with a TGF-β type II receptor (TβR-II) construct used as a negative control (data not shown).

Probing of a human kidney cDNA library with the BMPR-II cDNA obtained in the two-hybrid screen yielded three overlapping clones with a complete open reading frame predicting a 530-amino-acid protein with structural features typical of a type II receptor (Fig. 1A). These features include a short cysteine-rich extracellular domain, a transmembrane region, and a short segment preceding the kinase domain.

Receptor interactions in mammalian cells. The cloning of BMPR-II as a type I receptor-interacting protein suggested that it was either a receptor component that participates in type I receptor signaling or a novel type II receptor capable of nonselective interaction with type I receptor cytoplasmic domains when expressed in yeast cells. To test these possibilities, we first examined the ability of BMPR-II to interact with TGF-β, activin, and their receptors. BMPR-II was transfected alone or in combination with TGF-β or activin receptors in COS-1 cells, and the transfectants were sequentially incubated with ^{125}I-TGF-β1 or ^{125}I-activin A and disuccinimidyl suberate to cross-link receptors to bound ligands. The resulting receptor labeling patterns showed that unlike the TGF-β and activin type II receptors, BMPR-II was unable to bind TGF-β or activin either alone or in concert with the type I receptors TβR-I and ActR-IB (Fig. 2A).

To determine whether the interaction detected in yeast cells also took place in mammalian cells, the receptor cDNAs were modified to encode short C-terminal sequence tags that facilitate recovery of receptor complexes. TβR-II tagged with a hexahistidine sequence that binds to nickel-NTA-agarose (TβR-II^{His}) and TβR-I tagged with an influenza virus HA epitope (TβR-I^{HA}) (35) were cotransfected into R-1B/L17 mink lung epithelial cells, metabolically labeled, and incubated with TGF-β1. Receptor complexes were isolated by a two-step precipitation protocol with nickel-NTA-agarose and anti-HA–agarose. As previously reported (52), the results of this experiment showed that TGF-β induces association of these receptors (Fig. 2B). Notably, an analogous experiment with BMPR-II^{His} and TβR-I^{HA} provided no evidence for TGF-β-induced association of these two receptors (Fig. 2B).

Ligand-independent complexes were observed between fast-migrating forms of BMPR-II^{His} and TβR-I^{HA} (Fig. 2B); however, control metabolic labeling experiments showed that these forms are biosynthetic precursors (data not shown). This suggested that the association results from aggregation of the overexpressed proteins in the endoplasmic reticulum. Alternatively, it is conceivable that in the absence of some specific constraint imposed by correctly folded extracellular domains, the cytoplasmic domains of these receptors might interact in mammalian cells as they did in yeast cells. No specific association was detected between Flag epitope-tagged BMPR-II (BMPR-II^{Flag}) and cotransfected TβR-II^{His} or between BMPR-II^{Flag}, TβR-I^{HA}, and TβR-II^{His} in triple transfectants (Fig. 2B). Single precipitations demonstrated high expression of all transfected receptors (data not shown). Thus, despite its ability to interact with TβR-I in yeast cells, BMPR-II does not interact with this receptor or with the ligand-induced TβR-I/TβR-II complex in mammalian cells.

BMPR-II has no effect on TGF-β or activin receptor signaling, as determined by measuring activation of the p3TP-Lux reporter construct by these factors. The p3TP-Lux construct, which contains three TPA (tetradecanoyl phorbol acetate) response elements and a portion of the plasminogen activator inhibitor 1 promoter controlling expression of a luciferase gene (9, 51), responds to TGF-β and activin and provides an efficient system to test signaling by receptors transiently transfected in appropriate cells (8, 51). TβR-II transfection into the TβR-II-defective DR-26 mink lung epithelial cell line restores p3TP-Lux responsiveness to TGF-β (51), whereas BMPR-II transfection does not (Fig. 2C). Mutation of a conserved lysine in the ATP binding site of TβR-II to arginine disrupts kinase activity, yielding a dominant negative receptor that inhibits TGF-β responses when transfected into wild-type cells (48). However, when tested in this manner, the analogous BMPR-II

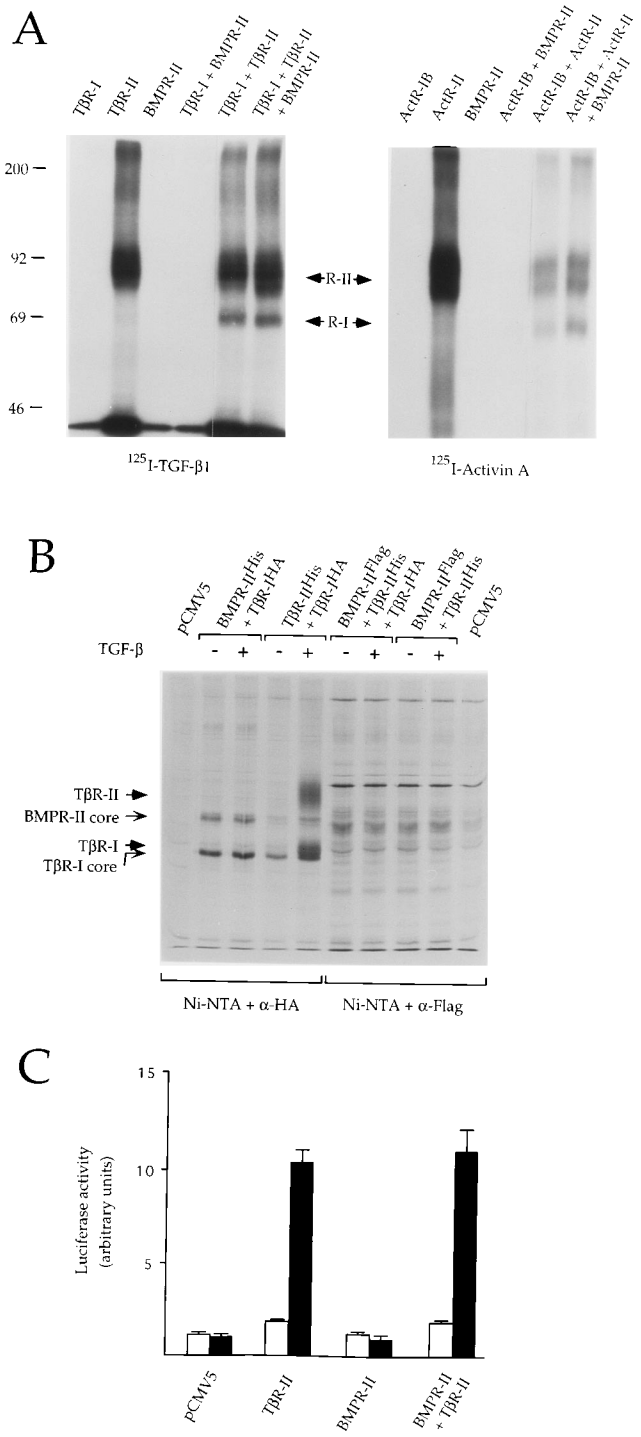

FIG. 2. Receptor interactions in mammalian cells. (A) Receptor-ligand interactions. COS-1 cells were transfected with the indicated receptor vectors and cross-linked to cell-bound ^{125}I-TGF-β1 or ^{125}I-activin A. Cell extracts were subjected to SDS-PAGE and autoradiography. Sizes are shown in kilodaltons. (B) Ligand-induced receptor complexes. R-1B/L17 cells were transfected with the indicated tagged receptors, metabolically labeled with [^{35}S]methionine, and incubated with or without 1 nM TGF-β1 for 15 min at 37°C. Receptor complexes were isolated from cell lysates by two-step precipitation with the agarose affinity beads indicated at the bottom of each panel and subjected to SDS-PAGE and autoradiography. (C) Reporter gene activation. DR-26 cells were transfected with p3TP-Lux and the indicated receptors and then incubated with (solid bars) or without (open bars) TGF-β1. Luciferase activity was assayed in triplicate samples and is plotted as the average ± standard deviation.

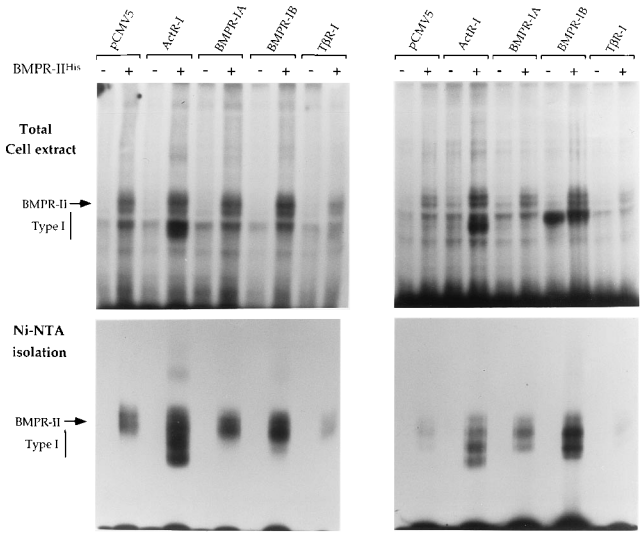

FIG. 3. BMP binding activity. COS-1 cells transfected with the indicated type I receptors alone (−) or together with BMPR-II^{His} (+) were labeled by incubation with ^{125}I-BMP-2 (right panel) or ^{125}I-BMP-7 (left panel) and a cross-linking agent. Cell extracts were subjected to SDS-PAGE and autoradiography directly (total cell extract) or after precipitation with Ni-NTA-agarose beads.

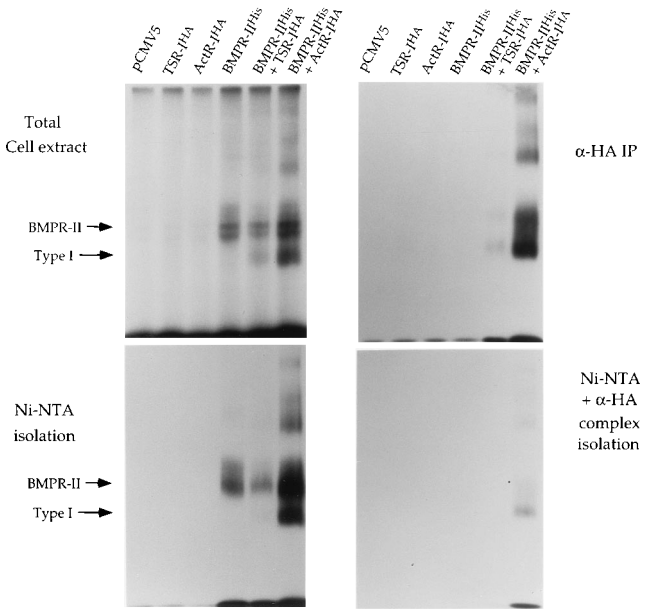

FIG. 4. BMP receptor complexes. COS-1 cells transfected with the indicated tagged receptor constructs were labeled by incubation with ^{125}I-BMP-2 and cross-linking. Cell extracts were subjected to SDS-PAGE and autoradiography directly (total cell extract) or after precipitation with Ni-NTA-agarose beads (Ni-NTA isolation), immunoprecipitation with HA antibody (α-HA IP), or a two-step precipitation to isolate receptor complexes (complex isolation).

mutant construct [BMPR-II(K230R)] did not affect the 3TP-Lux response to TGF-β or activin (data not shown).

BMP binding properties. BMP-2 and BMP-7 (also known as osteogenic protein-1 [OP-1] [37]) have 60% identity at the amino acid sequence level but represent two distinct subgroups within the TGF-β superfamily. BMP-2 is highly related to BMP-4 and to *Drosophila* Dpp, whereas BMP-7 is highly related to BMP-5, -6, and -8 and to *Drosophila* 60A factor (33). We used BMP-2 and BMP-7 as representatives of these two groups in binding assays. When incubated with ^{125}I-BMP-2 (Fig. 3, right panels) or ^{125}I-BMP-7 (Fig. 3, left panels) followed by a cross-linking agent, COS-1 cells transfected with BMPR-II^{His} alone yielded labeled products of 90 kDa whose identity as BMPR-II was confirmed by precipitation with Ni-NTA-agarose (Fig. 3).

We next tested the ability of BMPR-II to bind BMPs in concert with type I receptors. Three mammalian BMP type I receptors have been described, BMPR-IA (known as BRK-1 from mouse) (27), BMPR-IB, and ActR-I (45), the last being a mixed-specificity receptor that binds activin in concert with activin type II receptors (1, 44). In contrast to other type I receptors, which do not show detectable ligand-binding activity when expressed alone, BMPR-IA and -IB transfected alone can bind BMP-4 and BMP-7 to some extent under certain conditions (27, 45). ActR-I, BMPR-IA and -IB, and TβR-I and TSR-I as controls were transfected alone or together with BMPR-II^{His} into COS-1 cells, and the cells were labeled with ^{125}I-BMP-2 or ^{125}I-BMP-7. Analysis of labeled total cell extracts or after binding to Ni-NTA beads showed that several type I receptors increased BMP binding to BMPR-II (Fig. 3). Furthermore, BMPR-II coprecipitated with labeled products in the 70- to 85-kDa range that corresponds to the predicted size of the labeled type I receptors. The pattern of labeled products suggested that BMPR-II cooperated with ActR-I and BMPR-IB and to a lesser extent with BMPR-IA in binding BMP-2 (Fig. 3, right panels) and cooperated with ActR-I and to a lesser extent with BMPR-IA and -IB in binding BMP-7 (Fig. 3, left panels). Little or no BMP binding was detected to TβR-I (Fig. 3) or TSR-I (Fig. 4), and neither receptor enhanced BMP binding to BMPR-II. Precipitations from metabolically labeled cells demonstrated similar expression levels of all transfected receptors (data not shown).

To confirm that BMPR-II bound ligand cooperatively with a cotransfected type I receptor and formed a complex, an HA-tagged ActR-I construct was cotransfected with BMPR-II^{His}. Recovery of these receptors from ^{125}I-BMP-2-labeled cells showed that ActR-I and BMPR-II were more intensely labeled when expressed together than when expressed alone, and each receptor coprecipitated with a labeled species corresponding in size to the other receptor (Fig. 4). Elution of bound material from Ni-NTA beads with imidazole and precipitation of this eluate with anti-HA yielded both receptor products (Fig. 4), confirming their association. No significant BMP-dependent association was observed between cotransfected TSR-I and BMPR-II (Fig. 4).

Signaling activity in response to BMP. To determine whether BMPR-II and its associated type I receptors are capable of signaling upon binding BMP, we analyzed the ability of single receptors and receptor combinations to signal in cells. Since no specific readouts for BMP receptor signalling are currently available, we measured transcriptional activation of the reporter construct p3TP-Lux. R-1B/L17 cells transfected with p3TP-Lux and empty pCMV5 vector showed no luciferase response to BMP-2 or BMP-7 (Fig. 5, first set of bars), and therefore these cells were used for the analysis of transfected BMP receptor signaling activity. Cells transfected with BMPR-II or ActR-I alone did not respond to BMP-2 or BMP-7 (Fig. 5, second and third sets of bars); however, cells cotransfected with BMPR-II and ActR-I showed a threefold induction of luciferase activity in response to BMP-2 and a fivefold induction in response to BMP-7 (Fig. 5).

To test the dependence of these responses on the kinase activity of the two receptors, similar experiments were done with receptor constructs containing lysine-to-arginine muta-

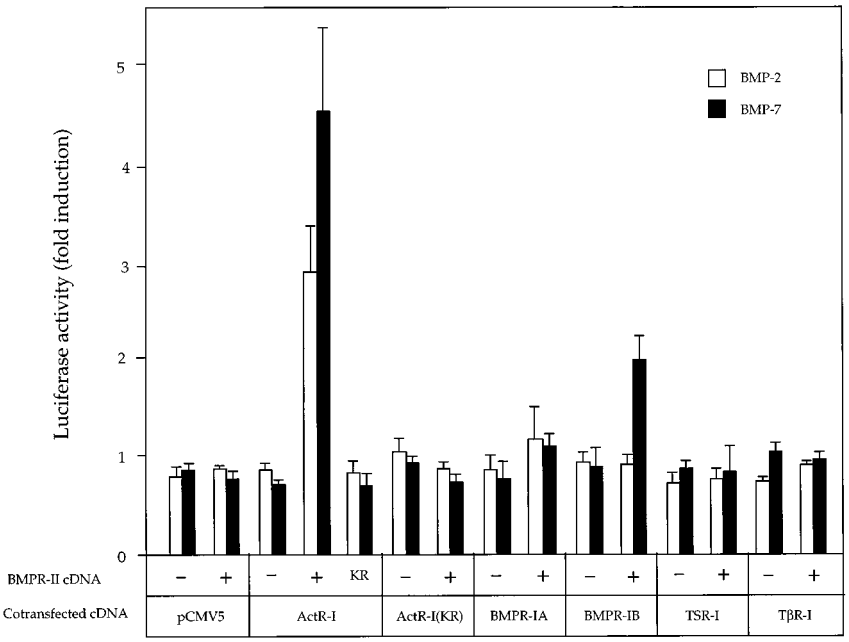

FIG. 5. BMP activation of receptor signaling. R-1B/L17 cells were transfected with p3TP-Lux and the type I receptor vectors indicated at the bottom, either alone (−) or together with BMPR-II (+). Where indicated (KR), the kinase-defective BMPR-II(K230R) and ActR-I(K235R) constructs were used. Cells were incubated with 2 nM BMP-2 (open bars) or 2 nM BMP-7 (solid bars) or no additions, and luciferase activity was assayed in triplicate cell lysates. Data are plotted as fold induction of luciferase activity relative to that in cells that received no additions and are the averages of results from three experiments ± standard deviation.

tions in the ATP binding site. Cells cotransfected with these constructs, BMPR-II(K230R) and ActR-I(K235R), and the wild-type version of the other receptor failed to respond to BMP-2 or BMP-7 (Fig. 5).

Cells cotransfected with BMPR-II and BMPR-IB showed a small response to BMP-7 but no response to BMP-2 (Fig. 5). Cells cotransfected with BMPR-II and BMPR-IA did not show a luciferase response to BMP-2 or BMP-7. TβR-I and TSR-I transfectants were also unresponsive to BMPs (Fig. 5), even though TβR-I mediates a strong luciferase response to TGF-β in these cells (6, 8). These results suggested that the response to BMP-2 and BMP-7 resulted from a specific interaction of these ligands with selected type I and type II receptor combinations.

DISCUSSION

BMPs are phylogenetically conserved and occupy a unique position in the TGF-β superfamily (illustrated in Fig. 6A). Progress in understanding the mode of signaling by TGF-β-related factors has been made with the identification of pairs of type I and type II receptor serine/threonine kinases that, upon binding TGF-β or activin, generate the first step of a signaling pathway (25, 33, 52). The interdependence of these two receptor types was first inferred from the inability of receptor I to bind ligand in the absence of receptor II and the inability of receptor II to signal in the absence of receptor I (51). However, it has been unclear whether this scheme also applies to the BMPs and their receptors. With the exception of Daf-4, whose ligand in the nematode is not known (14), no BMP type II receptor has been described previously. Furthermore, various human and *Drosophila* type I receptors can bind BMP in the absence of a type II receptor, raising the possibility that BMPs signals solely through type I receptors. The present identification of a human BMP type II receptor and the demonstration that BMP signaling in mammalian cells requires the cooperative interaction of type I and type II receptors provide an initial resolution of these questions.

The cloning of BMPR-II was achieved by the yeast two-hybrid system, using as bait the cytoplasmic domain of the TGF-β type I receptor. The use of this system to identify novel partners of TβR-I was based on the previous observation that the TβR-I and TβR-II cytoplasmic domains interact in the yeast two-hybrid system (46). However, this interaction is relatively weak, and therefore, it is surprising that TβR-I interacts strongly with the cytoplasmic domain of the heterologous type II receptor BMPR-II in yeast cells. Several lines of evidence suggest that TβR-I and BMPR-II are not physiological partners in mammalian cells. First, the mature, fully glycosylated forms of these receptors do not associate with each other in mammalian cells. Second, BMPR-II fails to bind TGF-β, support TGF-β binding to TβR-I, or associate with the ligand-induced TβR-I/TβR-II complex. Third, BMPR-II transfection does not rescue TGF-β responsiveness in TβR-II-defective cells. Fourth, TGF-β responsiveness in wild-type cells is not altered by transfection of wild-type BMPR-II or a kinase-defective BMPR-II construct. BMPR-II can indiscriminately interact with every type I receptor cytoplasmic domain tested in yeast cells, including TβR-I, ActR-I, ActR-IB, and TSR-I, but does not interact with the TβR-II cytoplasmic domain. This suggests that BMPR-II has affinity for a shared motif in type I receptors, perhaps the GS motif. However, in contrast to the interactions observed in yeast cells, BMPR-II expressed as a full-length protein in mammalian cells does not interact with TGF-β or activin receptors, but only with BMP type I receptors.

Our conclusion that BMPR-II is a human BMP type II receptor rests on its structural properties, ligand binding, and signaling activity. BMPR-II belongs to the type II receptor serine/threonine kinase family on the basis of its kinase domain sequence, lack of a GS domain, and presence of a C-terminal

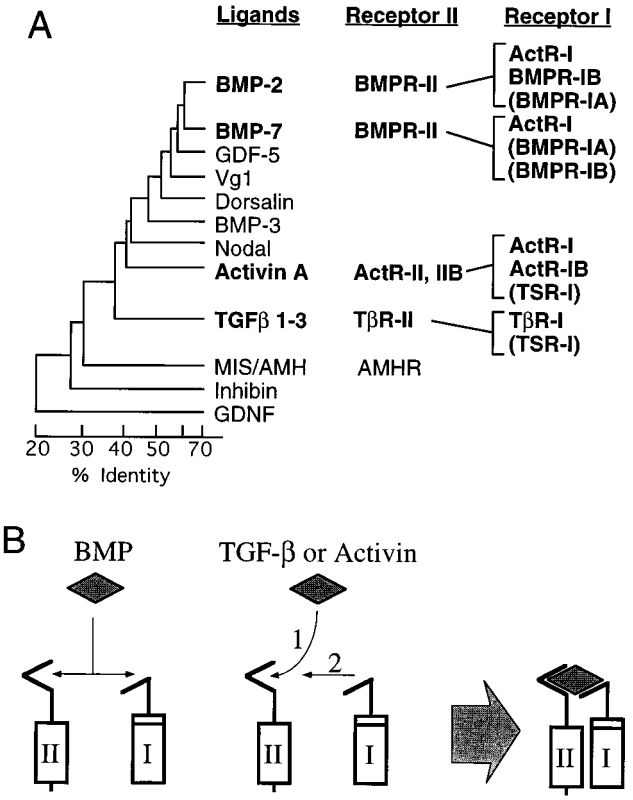

FIG. 6. Summary of receptor interactions in the TGF-β family in vertebrates. (A) Abbreviated TGF-β family dendrogram and receptor interactions. Most listed TGF-β family members each represent a cluster of closely related (>75% identical) isoforms (33). All members are from mammals except chicken dorsalin and *Xenopus* Vg1. Type II receptors are shown with their known type I receptor partners. Receptor interactions that are weak or without known signaling activity are indicated in parentheses. (B) Models of ligand interaction with heteromeric kinase complexes. Most BMP receptor interactions identified in the present studies are cooperative, requiring BMP receptors I and II for maximal ligand binding. In contrast, previously identified TGF-β and activin receptor interactions suggest that ligand binds primarily to receptor II, with subsequent recognition by receptor I, which cannot bind ligand on its own.

extension typical of type II receptors (3, 36). BMPR-II transfected alone in test cells binds BMP-2 and BMP-7 but not TGF-β1 or activin A. Furthermore, expression of BMPR-II enhances or is essential for BMP binding to ActR-I, BMPR-IA, and BMPR-IB. However, unlike ligand binding to type II receptors for TGF-β and activin, binding to BMPR-II is enhanced by coexpression of BMP type I receptors. This phenomenon argues that BMP binding to these receptors is cooperative and suggests that BMP receptors I and II jointly form a high-affinity binding site. This ligand-binding scheme is intermediate between the binding pattern of TGF-β and activin receptors (Fig. 6B) and the binding patterns of various members of the cytokine receptor superfamily (26, 42).

Using the same cloning approach as described here, Kawabata et al. (23) recently cloned a serine/threonine kinase receptor with a ubiquitous pattern of expression of three distinct messages. Comparison of the sequences revealed that this receptor is identical to BMPR-II except that it has a long C-terminal extension that might arise from an alternative splicing event. The functional properties of this receptor form were not determined, but on the basis of the present results, it is likely that this receptor form also binds BMPs.

Depending on the ligand, BMPR-II differs in its ability to interact with three mammalian type I receptors, and the resulting receptor complexes differ in signaling capacity. Under our assay conditions, BMP-2 binds better to BMPR-II in combination with ActR-I or BMPR-IB than in combination with BMPR-IA, whereas BMP-7 binds better to BMPR-II in combination with ActR-I than in combination with BMPR-IA or -IB (see summary in Fig. 6A). Acting together in response to ligand binding, BMPR-II and ActR-I but not the other receptor combinations mediate activation of the p3TP-Lux reporter gene construct. Human ActR-I was previously identified as a type I receptor that binds activin in concert with ActR-II or ActR-IIB (1, 44) and mediates this same response (1). Thus, ActR-I can act as both an activin type I receptor and a BMP type I receptor, depending on what partners are available in the cell. These results need to be refined by determining the binding properties of these receptor combinations in cells expressing physiological levels of receptors, by testing other members of the TGF-β family as potential ligands as they become available in recombinant form, and, above all, by in vivo studies to determine the physiological roles of these receptors.

We have demonstrated that the signaling activity of a BMP receptor complex depends on the kinase activity of both receptor components, a phenomenon previously observed with TGF-β receptors (6, 8, 16, 22, 51) and activin receptors (4). In these systems, the type II receptor acts as the primary receptor, and the type I receptor acts as its substrate and downstream signaling component (4, 52). It has been proposed that the two receptor types in the complex mediate separate responses (11); however, this proposal is not tenable, since a constitutively active form of the TGF-β type I receptor can mediate both types of responses independently of ligand or type II receptor (49). Likewise, the inability of a BMP type I receptor to signal in the absence of a coexpressed type II receptor argues against the notion that the limited binding of BMPs to type I receptor can generate a signal. The requirement for a heteromeric receptor complex for signaling by BMP receptors establishes the generality of this model in the TGF-β receptor family.

The general properties of the human BMP receptor system described here are remarkably similar to those of recently identified Dpp receptors from *D. melanogaster* (28, 40). In both systems, a type II receptor (BMPR-II in humans and Punt in *Drosophila* cells) has a choice of several type I receptors (ActR-I and BMPR-IA or -IB in humans, Thickveins or Saxophone in *Drosophila* cells), and the interaction between the two receptor types is essential for signaling. In both systems, ligand binding is cooperative between type I and type II receptors instead of being determined solely by the type II receptors. Finally, ActR-I from humans and Punt from *Drosophila* cells can bind both BMP-related factors and activin-related factors, and therefore, both systems have the potential for activin-BMP crosstalk. As recently suggested by others (19, 24) and illustrated by the present results, the combinatorial nature of these receptors may explain their involvement in diverse biological processes and should be taken into consideration when analyzing their role in such processes.

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