Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 10255–10259, October 1994
Developmental Biology

A truncated bone morphogenetic protein receptor affects dorsal–ventral patterning in the early *Xenopus* embryo

ATSUSHI SUZUKI*, R. SCOTT THIES†, NOBORU YAMAJI†‡, JEFFREY J. SONG†, JOHN M. WOZNEY†, KAZUO MURAKAMI§, AND NAOTO UENO*¶

*Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan; †Genetics Institute Inc., 87 Cambridge Park Drive, Cambridge, MA 02140; ‡Yamanouchi Pharmaceutical Co., Ltd., Tokyo 103, Japan; and §Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

Communicated by Igor B. Dawid, July 13, 1994

ABSTRACT **Bone morphogenetic proteins (BMPs), which are members of the transforming growth factor β (TGF-β) superfamily, have been implicated in bone formation and the regulation of early development. To better understand the roles of BMPs in *Xenopus laevis* embryogenesis, we have cloned a cDNA coding for a serine/threonine kinase receptor that binds BMP-2 and BMP-4. To analyze its function, we attempted to block the BMP signaling pathway in *Xenopus* embryos by using a dominant-negative mutant of the BMP receptor. When the mutant receptor lacking the putative serine/threonine kinase domain was expressed in ventral blastomeres of *Xenopus* embryos, these blastomeres were respecified to dorsal mesoderm, eventually resulting in the formation of a secondary body axis. These findings suggest that endogenous BMP-2 and BMP-4 are involved in the dorsal–ventral specification in the embryo and that ventral fate requires induction rather than resulting from an absence of dorsal specification.**

Pattern formation in vertebrate embryos involves the specification of regional cell fates along the two major body axes, the anterior–posterior and dorsal–ventral axes. The establishment of these body axes is believed to require various signaling molecules that regulate inductive cell–cell interaction as well as autonomous cell differentiation. Recent studies in the African clawed frog, *Xenopus laevis*, have suggested that diffusible polypeptide growth factors play fundamental roles in dorsal–ventral patterning of the early embryo. Activin, a member of the transforming growth factor β (TGF-β) superfamily, has been studied intensely since it was found to induce dorsal mesoderm in animal cap ectoderm explants (1). An important issue regarding the function of growth factors in early development is whether the protein is present at the relevant tissue and place. The mRNA for activin and an activin-like biological activity have been detected during *Xenopus* embryogenesis (2, 3). *Xenopus* activin receptor genes, which encode transmembrane proteins possessing the intracellular serine/threonine kinase domains, have been cloned recently, and activin signaling pathways via these receptors have been implicated in mesoderm induction and dorsal axis formation (4, 5). In addition, the function of endogenous activin was addressed by using a dominant-negative form of activin receptor that lacks the serine/threonine kinase domain. Overexpression of the mutant activin receptor in the early embryo blocked the formation of mesoderm, suggesting that endogenous activin is essential for mesoderm induction (6).

In addition to activin, bone morphogenetic proteins (BMPs), originally identified in bovine bone, have been implicated in various morphogenetic processes such as bone formation, neurogenesis, and epithelial–mesenchymal interactions (7). The transcripts for BMP-2 and -4 as well as the corresponding proteins are present in developing *Xenopus* embryos, and overexpression of BMP-4 in the embryos enhances the formation of ventral mesoderm (8–11). Animal cap ectoderm treated with a combination of BMP-4 and activin also results in the formation of ventral mesoderm, suggesting that BMP-4 is a ventralizing factor that acts by overriding the dorsalizing signal provided by activin (8, 9). Therefore, activin and BMP-4 are thought to play important roles in the dorsal–ventral patterning of embryonic mesoderm. Although the exogenously applied BMP has been used to address the functions of the ligand in the embryo, little is known about the involvement of endogenous BMP ligands in dorsal–ventral patterning of the mesoderm.

Here we report the isolation of a vertebrate serine/threonine kinase receptor cDNA whose product displays specific binding to BMP-2 and BMP-4.‖ In addition, we were able to block the BMP signaling pathway in early *Xenopus* embryos by using a functionally negative mutant of the BMP receptor. Overexpression of the negative receptor in ventral blastomeres of *Xenopus* embryos resulted in duplication of the body axis by respecifying the presumptive ventral mesoderm to dorsal mesoderm. The data presented here strongly suggest that specification of the dorsal–ventral axis of *Xenopus* embryo is controlled by endogenous BMP present in the embryo.

MATERIALS AND METHODS

Cloning of a Mouse BMP Receptor. A set of degenerate oligonucleotide primers was designed in serine/threonine kinase subdomains VIII and XI (12), based on amino acid sequences of mouse activin type II receptor (13) and human TGF-β type II receptor (14). The forward polymerase chain reaction (PCR) primer (domain VIII) was 5′-TA(TC)ATGGC-(TCAG)CC(TCAG)GA(AG)GT-3′; the reverse PCR primer (domain XI) was 5′-(AG)TC(AG)TG(AG)TCCCA(AG)-CA(TC)TC-3′. Total RNA was purified from the mouse MC3T3-E1 cells and subjected to reverse transcription PCR (RT-PCR) as described (15). Forty cycles of amplification were performed, each consisting of denaturation at 94°C for 1 min, annealing at 45°C for 1 min, and extension at 72°C for 2 min. The amplified PCR products were subcloned into pBluescript II KS (−) and sequenced using Sequenase (United States Biochemical). One of the clones, designated as mTFR11, was used as a probe for screening the MC3T3-E1 cell cDNA library and mouse brain cDNA library, resulting in the isolation of a clone that encoded a 532-amino acid protein.

Binding Assay and Affinity Cross-Linking. Human TGF-β1 was purchased from R&D Systems. Human activin A is a gift from Y. Eto and H. Shibai (Ajinomoto). Human BMP-2 and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: TGF-β, transforming growth factor β; BMP, bone morphogenetic protein; RT-PCR, reverse transcription polymerase chain reaction; DAI, dorsoanterior index.

¶To whom reprint requests should be addressed.

‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. D16250).

-4 were purified from Chinese hamster ovary cell lines (16). To express mTFR11 in COS cells, full-length cDNA of mTFR11 was subcloned into pMV2, which is a derivative of pMT2 (17). Human recombinant BMP-4 was iodinated by the methods of Frolik *et al.* (18). The iodinated BMP-4 exhibited biological activity similar to that of unlabeled BMP-4 (data not shown) in a W-20-17 cell (16). Transfected COS cells were incubated with BMP-4 and/or ^{125}I-labeled BMP-4 (^{125}I-BMP-4) at 37°C for 60 min. Following binding, the cells were washed twice and radioactivity was determined in a γ counter. In the competition assay, COS cells transfected with mTFR11 were incubated with ^{125}I-BMP-4 with or without increasing concentrations of unlabeled BMP-2, BMP-4, activin A, or TGF-β1. Results are expressed as means for triplicate wells from one experiment and were confirmed in a second independent experiment. For cross-linking studies, the cells were incubated with 500 μM disuccinimidyl suberate for 20 min at 4°C following binding. Cell extracts were analyzed on SDS/PAGE under reducing conditions.

Microinjection of Synthetic mRNAs and Histological Analysis. A dominant-negative mutant of mTFR11 was constructed by using one of 3′-nested deletion clones of mTFR11 cDNA. This 3′-deleted cDNA has a termination codon instead of Tyr at amino acid 176 and codes for the extracellular domain and transmembrane domain of mTFR11. The truncated and a full-length cDNAs of mTFR11 were subcloned into pSP64T (19). Synthesis and microinjection of capped mRNA were performed essentially as described by Moon and Christian (20). Embryos were staged according to Nieuwkoop and Faber (21). The injected embryos were allowed to develop until stages 34–40 for the observation of external appearance and then subjected to histological analysis. Whole-mount *in situ* hybridization was performed as described by Harland (22).

RESULTS

Identification of a Vertebrate BMP-2/-4 Receptor. To better understand the molecular basis of BMP functions in early *Xenopus* embryos, we isolated cDNA encoding a BMP receptor. Since recent isolation of type I and type II activin and TGF-β receptors has revealed a conserved intracellular serine/threonine kinase domain (23), we employed the RT-PCR using degenerate oligonucleotide primers in serine/threonine kinase subdomains VIII and XI (12). From total RNA isolated from mouse MC3T3-E1 cells, a cell line known to be highly sensitive to BMP-2 and BMP-4 (24, 25), mTFR11 clone was isolated. mTFR11 cDNA codes for a 532-amino acid receptor-like protein consisting of a 19-amino acid hydrophobic signal sequence, a 133-amino acid extracellular ligand binding domain, a 24-amino acid transmembrane domain, and a 356-amino acid cytoplasmic serine/threonine kinase domain (Fig. 1*A*). There are three potential N-glycosylation sites, one in the extracellular domain and two in the cytoplasmic domain. The receptor has a typical amino acid sequence (-GSGSGLPXXVQRT-), called type I box, within the juxta-membrane domain. This domain is conserved among previously reported type I receptors for factors in the TGF-β superfamily (23). The homology of each functional domain of the mTFR11 receptor to other reported serine/threonine kinase receptors is illustrated in Fig. 1*B*. Although the mTFR11 is similar to ALK-3 and ALK-6, whose specific ligand is unknown (26), the mTFR11 is most similar to ALK-3 (98%), indicating that the mTFR11 receptor is a mouse homologue of human ALK-3. In addition, a *Xenopus* homologue of mTFR11 has been cloned and the amino acid sequence identity of the kinase domain indicates the receptor belongs to the mTFR11/hALK-3 subfamily. Furthermore, mTFR11 has overall 60% amino acid homology with the type I receptor for activin or TGF-β in the intracellular kinase domain but shows less homology with *daf-4*, a recently

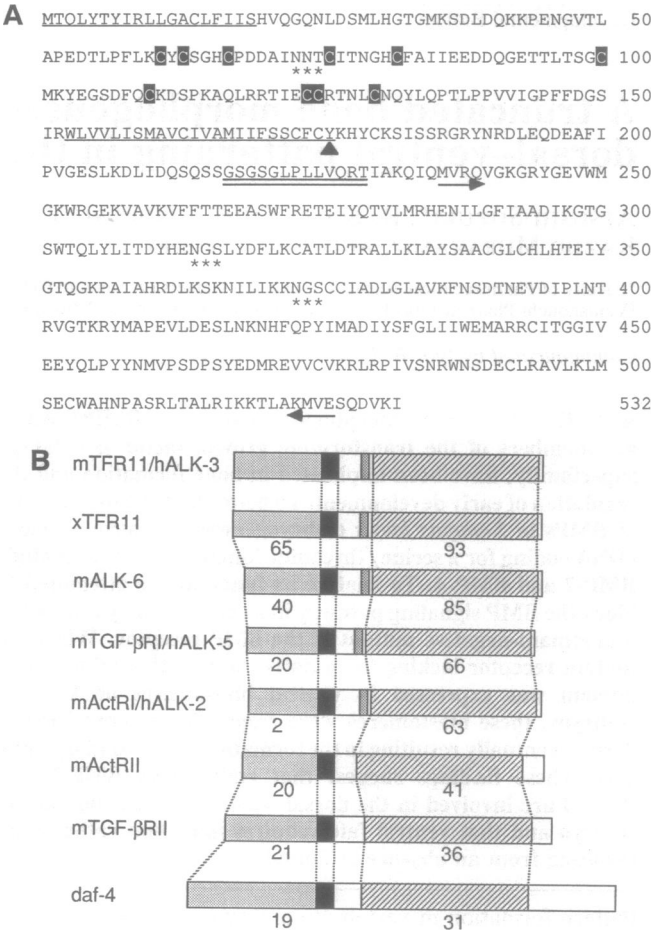

FIG. 1. Amino acid sequence and homology of mTFR11. (*A*) Translated amino acid sequence of the mTFR11 cDNA. The signal peptide and transmembrane domains are indicated by a single underline. Potential sites of N-linked glycosylation are indicated by asterisks. The ends of the kinase domain are indicated by arrows. Ten of the conserved cysteine residues in the extracellular domain are in reverse print. Type I box is indicated by a double underline. An arrowhead is under the tyrosine residue that changes to stop codon in the dominant negative BMP receptor. (*B*) Homology of the mTFR11/hALK-3 to other receptors for ligands of the TGF-β superfamily. Percent amino acid identity is indicated. Light-shaded boxes, extracellular domain; black boxes, transmembrane domain; dark-shaded boxes, type I box; hatched boxes, intracellular kinase domain. ALK-2, -3, -5, and -6, activin receptor-like kinases 2, 3, 5, and 6 (26); xTFR11, *Xenopus* TFR11 (accession no. D32066); mTGF-βRI, mouse TGF-β type I receptor (27); mActRI, mouse activin type I receptor (28); mActRII, mouse activin type II receptor (13); mTGF-βRII, mouse TGF-β type II receptor (29); daf-4, *C. elegans daf-4* gene product (30).

isolated BMP receptor from *Caenorhabditis elegans* belonging to the type II class of the receptor family (30).

To test the ability of mTFR11 to bind BMPs, COS cells transiently transfected with mTFR11 were incubated with iodinated recombinant human BMP-4. In Fig. 2*A*, the binding of ^{125}I-BMP-4 to mTFR11 expressed in COS cells is specific since it is displaced by an excess amount of unlabeled BMP-4. In contrast, COS cells transfected with vector alone showed very little specific BMP-4 binding, consistent with low levels of endogenous receptors in this cell line. Scatchard analysis of the binding data shown in Fig. 2*A Inset* indicated a single class of binding sites with a K_d of 3.5 nM. In competition assays, BMP-2, but not TGF-β1 or activin A, displaced the binding of BMP-4, suggesting that mTFR11 receptor binds BMP-2 and BMP-4 specifically (Fig. 2*B*). xTFR11 has also been shown to have the same ligand binding specificity (data not shown). The

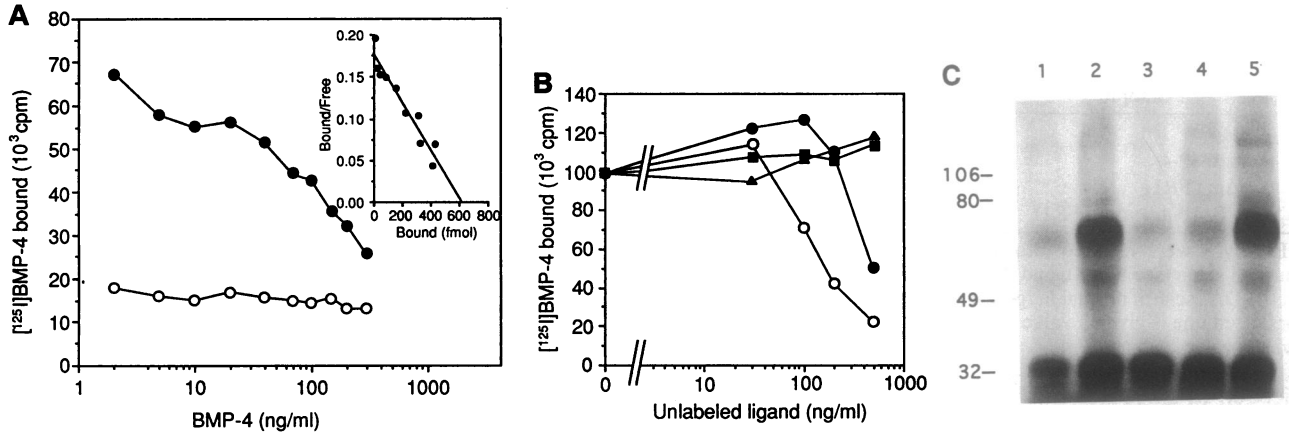

FIG. 2. Specific binding of iodinated BMP-4 to COS cells transfected with mTFR11. (*A*) ^{125}I-BMP-4 binding to COS cells transfected with mTFR11. Binding was performed on cell monolayers as described below and was competitively inhibited with unlabeled BMP-4. (*Inset*) Scatchard analysis. ○, pMV2; ●, mTFR11/pMV2. (*B*) Specificity of ^{125}I-BMP-4 binding to COS cells transfected with mTFR11. Binding of ^{125}I-BMP-4 to COS cells transfected with mTFR11 was performed with or without increasing concentrations of unlabeled ligands. ●, BMP-2; ○, BMP-4; ■, activin A; ▲, TGF-β1. (*C*) Chemical cross-linking of iodinated BMP-4 to COS cells transfected with mTFR11/pMV2 or pMV2. COS cells were transfected with either pMV2 (lane 1) or mTFR11/pMV2 (lanes 2–5) and incubated with 5 ng of ^{125}I-BMP-4 per ml (all lanes) and 500 ng of BMP-4 per ml (lane 3), 500 ng of BMP-2 per ml (lane 4), or 500 ng of activin per ml (lane 5). Molecular masses are indicated in kDa.

apparent molecular mass of the BMP-4-receptor complex was 70 kDa based on the results of chemical cross-linking experiments (Fig. 2*C*). By subtracting the molecular mass of monomeric BMP-4, a molecular mass of 55 kDa is predicted for mTFR11. These findings strongly suggest that mTFR11 encodes a vertebrate receptor for BMP-2 and -4.

Truncated BMP-2/-4 Receptor Neutralizes the Ventralizing Activity of BMP-4 in *Xenopus* Embryos. Previous studies showed that overexpression of truncated receptors for activin or TGF-β specifically inhibits the corresponding signaling pathway (6, 31). Therefore we generated a mutant BMP receptor (ΔmTFR11) that lacks the entire intracellular kinase domain and tested whether it could inhibit the BMP signaling pathway in early *Xenopus* embryos. Since overexpression of BMP-4 in the dorsal blastomeres causes ventralization (8, 9), we assessed the ability of the truncated receptor to inhibit this ventralizing activity in an mRNA injection experiment; the resulting phenotypes were scored using the dorsoanterior index (DAI) (32). The results in Fig. 3 and Table 1 show that the formation of dorsal mesoderm was inhibited (average DAI, 0.4) when BMP-4 mRNA was injected into the dorsal blastomeres at the four-cell stage. In contrast, coinjection of BMP-4 and ΔmTFR11 mRNAs resulted in normal axial structures such as the eyes, neural tube, somites, and notochord, leading to embryos with an average DAI of 4.9. Embryos injected into dorsal blastomeres with ΔmTFR11 mRNA alone did not show any defect in the patterning of the body axes. These findings indicated that the exogenous truncated BMP receptor in the *Xenopus* embryo can inhibit the BMP signaling pathway in a dominant-negative manner.

Inhibition of the Endogenous BMP-2/-4 Signaling Pathway. To examine the biological function of endogenous BMPs in dorsal–ventral specification, we attempted to block the signaling pathway by using a dominant-negative mutant of the BMP receptor. A current model suggests that BMP-4 is a ventralizing factor, and thus the BMP signal may be expected to be present in the vegetal ventral blastomeres. Consistent with this notion, injection of the mutant BMP receptor into the dorsal blastomeres did not affect dorsal–ventral axis formation (see Fig. 3 and Table 1). mRNAs encoding wild-type mTFR11 or ΔmTFR11 were injected into the marginal region of the ventral blastomeres of stage 3 (four-cell) embryos, and the resulting phenotypes were analyzed. Embryos injected with either anti-sense ΔmTFR11 mRNA (Fig. 4*A*) or distilled H_2O developed with no significant abnormalities (Table 2). Injection of sense mRNA encoding ΔmTFR11 into ventral blastomeres resulted in bifurcation of the neural tube (Fig. 4*B*) and eventual formation of a secondary body axis (Fig. 4*C*) at a frequency up to 76% (Table 2). The secondary axes induced by ΔmTFR11 lacked anterior structures such as a cement gland and eyes. In addition, the axis-inducing activity ΔmTFR11 mRNA depends on the amount of mRNA injected (Table 2). The duplication of the body axis caused by ΔmTFR11 injection was rescued by injection of an equal amount of sense mTFR11 mRNA, supporting the view that the phenotype is a specific effect of ΔmTFR11 (Table 2). Injection of wild-type mTFR11 mRNA (500 pg) into dorsal blastomeres or ventral blastomeres caused no significant changes in dorsal–ventral patterning as judged by external appearance (data not shown). Frequency with which secondary body axis was observed following

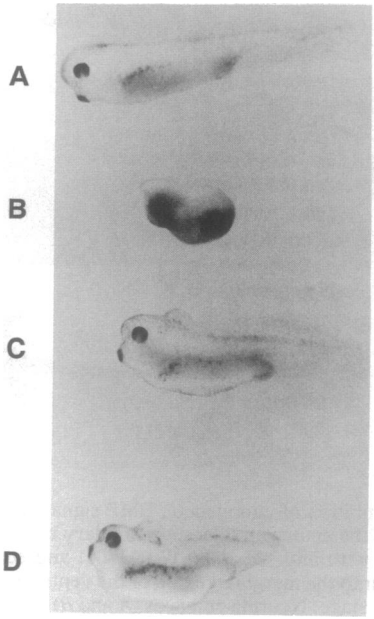

FIG. 3. Truncated BMP receptor inhibits BMP signaling pathway in early *Xenopus* embryo. At the four-cell stage, embryos were injected with the indicated samples into the dorsal marginal region and allowed to develop until stage 37 or 38. (*A*) Distilled H_2O. (*B*) BMP-4 mRNA, 200 pg. (*C*) ΔmTFR11 mRNA, 200 pg. (*D*) BMP-4 plus ΔmTFR11 mRNA, 200 pg of each.

Table 1. Truncated BMP receptor inhibits BMP signaling pathway

Sample	No. of embryos	Average DAI
H_2O	29	5.0
xBMP-4	30	0.4
ΔmTFR11	22	4.9
xBMP-4 + ΔmTFR11	28	4.1

Embryos were injected into the dorsal equatorial region at the four-cell stage (see Fig. 3), and the DAI was scored at stage 37 or 38. On the DAI scale a normal tadpole is given a value of 5, a completely dorsalized one is given a value of 10, and a totally ventralized one is given a value of 0.

injection of ΔxTFR11 mRNA was ≈67% and the structure lacking anterior region was indistinguishable from ΔmTFR11 injection (data not shown).

Embryos with secondary axes were analyzed histologically by hematoxylin/eosin staining, showing that neural tissue and muscle had been induced in the secondary structures (Fig. 4*D*). To confirm the results of the histological analysis, expression of the neural marker gene, neural cell-adhesion molecule (Fig. 4*E*), and the muscle marker gene, α-actin (Fig. 4*F*), in the secondary axis was demonstrated by whole-mount *in situ* hybridization. Both mRNA markers were detected in the secondary body axis, confirming that muscle and neural tissue were present in the induced structures. However, notochord was rarely observed in the secondary axes. These findings imply that BMP-4 signaling is critical for ventral mesoderm development and that inhibition of BMP-4 signaling causes dorsalization of mesoderm.

Inhibition of the BMP-2/-4 Signaling Pathway Affects Expression of Early Mesodermal Markers. To understand the molecular mechanism underlying the respecification of ventral to dorsal mesoderm in the embryo injected with ΔmTFR11 mRNA, expression of early mesodermal markers (*goosecoid*, *Xpo*, and *Xbra*) was analyzed in such embryos by whole-mount *in situ* hybridization. Typical expression patterns of these molecular markers in wild-type and dominant negative receptor mRNA-injected embryos are shown in Fig. 5. *goosecoid* is a transcription factor expressed in Spemann's organizer in gastrulae (33) and thus serves as a suitable marker for the most dorsal mesoderm. When embryos were injected with ΔmTFR11 in the ventral blastomeres, no ectopic expression of *goosecoid* was observed in the ventral marginal region, suggesting that the ectopically induced mesoderm is distinct from the organizer field (Fig. 5 *A* and *B*). Conversely, the *Xpo* gene (34), whose expression is closely correlated with the ventrolateral region of the gastrula marginal zone (Fig. 5*C*), was down-regulated by inhibiting the BMP signaling pathway (Fig. 5*D*). The *brachyury* gene (35), a pan-mesodermal marker, is normally expressed in control (Fig. 5*E*) and injected embryos (Fig. 5*F*), suggesting that injection of the dominant-negative BMP receptor mRNA does not inhibit general mesoderm formation. On the basis of these results, we conclude that inhibition of the BMP signaling pathway changes ventral to dorsal mesoderm, without affecting the induction of mesoderm in general.

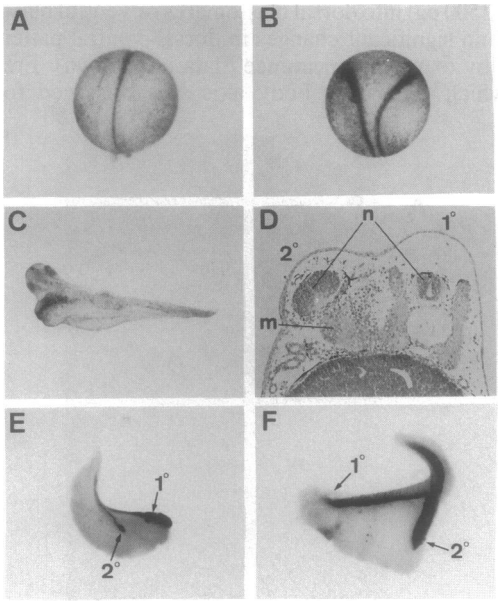

FIG. 4. Inhibition of endogenous BMP signaling pathway in the ventral part of the embryo induces a secondary body axis. Embryos were injected with anti-sense ΔmTFR11 (*A*) and sense ΔmTFR11 (*B–F*) mRNA into the marginal region of the ventral two blastomeres at the four-cell stage. Neurula embryos (*A* and *B*) show the formation of two neural tubes in the embryo injected with sense ΔmTFR11 mRNA. Tadpole stage embryo (*C*) and *D* show the secondary axial structures (n, neural tube; m, muscle); primary (1°) and secondary (2°) dorsal axes are indicated. Whole-mount *in situ* hybridization analysis of duplicate embryos shows induction of neural cell-adhesion molecule (*E*) and α-actin (*F*) mRNA in primary (1°) and secondary (2°) body axes.

Table 2. Phenotype of embryos injected with truncated BMP receptor mRNA

RNA injected, pg			Phenotype, %		
ΔmTFR11	mTFR11	No.*	N	SA	Defects†
0	—	44/37	88	0	5
20	—	58/51	52	27	9
200	—	58/46	7	76	2
200	200	54/54	91	0	6
400‡		54/50	89	0	0

Embryos were injected with synthetic mRNAs at the four-cell stage as described in the legend to Fig. 4 and analyzed at stage 33 or 34. N, normal; SA, secondary dorsal axis (see Fig. 4 and text).
*Total number of embryos/number swimming at stage 33 or 34.
†Includes posterior defects and incomplete invagination.
‡Anti-sense.

DISCUSSION

mTFR11 Encodes a Vertebrate BMP-2/-4 Receptor. The BMP receptor isolated from a mouse osteoblastic cell line encodes a protein with an intracellular serine/threonine kinase domain and belongs to the type I class of serine/threonine kinase receptors. In the case of TGF-β, three types of receptors have been identified in affinity cross-linking experiments, and type I and type II receptors are known to be transmembrane serine/threonine kinase proteins. It has been reported that the type I class of activin and TGF-β receptors requires their respective type II class receptors for ligand binding (23). Therefore, it is noteworthy that the transiently expressed mTFR11 receptor is capable of binding BMP-2 and -4 by itself, unless an endogenous BMP type II receptor, whose structure is yet unknown, exists in the COS cells. This matter may be elucidated in the future if the vertebrate homologue to *daf-4* (30), a BMP type II receptor isolated from *C. elegans*, is identified.

Role of Endogenous BMP-2/-4 Ligand in the Dorsal–Ventral Patterning of *Xenopus* Embryos. Blocking of the endogenous activin signaling pathway by using a negative activin receptor mutant is known to inhibit dorsal and ventral mesoderm formation (6). In contrast, disruption of the BMP signaling pathway in early *Xenopus* embryos using a negative BMP receptor mutant causes duplication of the body axis. It is interesting to note that inhibition of the endogenous BMP signaling pathway does not result in a loss of mesoderm but changes the fate of cells from the ventral marginal region to

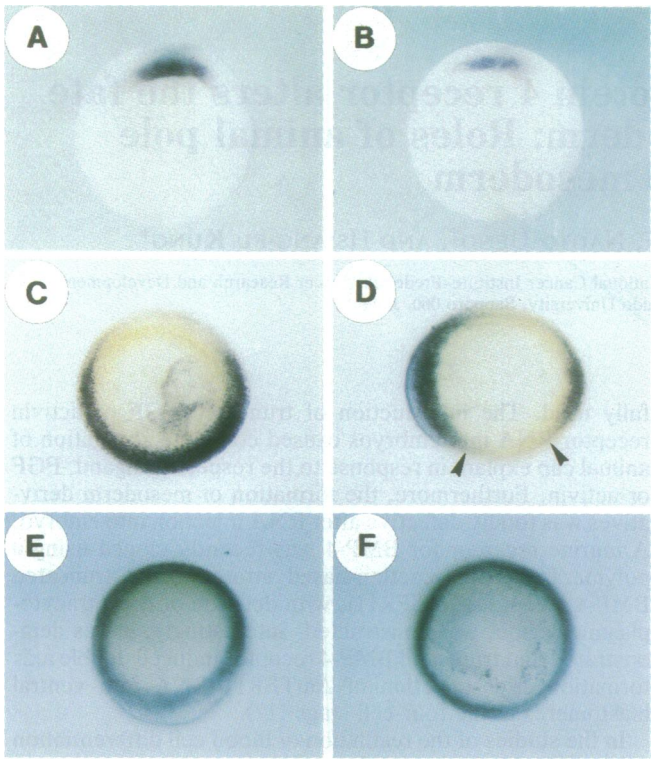

FIG. 5. Effect of inhibition of BMP signaling pathway on expression of early mesodermal markers. ΔmTFR11 mRNA was injected into the marginal region of ventral blastomeres at the four-cell stage. Uninjected control embryo (*A*, *C*, and *E*), and ΔmTFR11-injected embryos (*B*, *D*, and *F*) were developed until early gastrula stage and subjected to whole-mount *in situ* analysis. The probe for *goosecoid* was used in *A* and *B*; the probe for *Xpo* was used in *C* and *D*; the probe for *Xenopus brachyury* was used in *E* and *F*. Views of the vegetal pole are shown and dorsal is up. Note that the expression of *Xpo* gene disappeared in the ventral part of embryo that received truncated BMP receptor mRNA (arrowheads) but that of *brachyury* gene, normally expressed through mesoderm, was not affected.

form more dorsal mesoderm. Therefore, we postulate that endogenous BMP may act as a competence modifier rather than a mesoderm-inducing factor. Competence modifiers are factors that modify the response of tissue to mesoderm-inducing factors (36). We suggest that endogenous BMP cooperates with mesoderm-inducing factors and is critical for the formation of ventral mesoderm. Our analysis using a negative mutant of the BMP receptor shows that inhibition of the BMP signaling pathway altered the character of ventral mesoderm to a more dorsalized one. However, we were unable to show any ectopic activation of *goosecoid* gene to detectable levels by whole-mount *in situ* hybridization. This observation may be explained by the concept that in order to activate a full spectrum of genes expressed in dorsal mesoderm, a *noggin*-like dorsal competence modifier (37) is required. The identification of a vertebrate BMP receptor described here may provide new insight for understanding the crosstalk of cytoplasmic signals that trigger the sequential activation of early mesodermal genes. Further investigation is required to understand the signaling crosstalk between BMP and other factors, including activin, fibroblast growth factor, and *noggin*.

We thank Dr. M. Noda for providing mRNA from mouse MC3T3-E1 cells; Dr. K. Nakayama and M. Hosaka for providing a mouse brain cDNA library; Drs. I. Dawid, K. Cho, G. Thomsen, J. Smith, K. Miyazono, H. Ichijo, S. Nishimatsu, M. Asashima, M. Tada, and M. Yuge for helpful discussions; R. Zollner, B. Costigan, S. Dansereau, and B. Foster for providing the recombinant BMPs; and T. Yamagishi and N. Shioda for technical assistance. This work was supported by research grants from the Ministry of Education, Science and Culture, Japan (N.U., 03833001), Uehara memorial foundation (N.U.), and the Chichibu Cement Co. (K.M.). A.S. is a Research Fellow of the Japan Society for the Promotion of Science.

1. Sive, H. L. (1993) *Genes Dev.* **7**, 1–12.
2. Thomsen, G., Woolf, T., Whitman, M., Sokol, S., Vaughan, J., Vale, W. & Melton, D. A. (1990) *Cell* **63**, 485–493.
3. Asashima, M., Nakano, H., Uchiyama, H., Sugino, H., Nakamura, T., Eto, Y., Ejima, D., Nishimatsu, S., Ueno, N. & Kinoshita, K. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6511–6514.
4. Kondo, M., Tashiro, K., Fujii, G., Asano, M., Miyoshi, R., Yamada, R., Muramatsu, M. & Shiokawa, K. (1991) *Biochem. Biophys. Res. Commun.* **181**, 684–690.
5. Mathews, L. S., Vale, W. W. & Kintner, C. R. (1992) *Science* **255**, 1702–1705.
6. Hemmati-Brivanlou, A. & Melton, D. A. (1992) *Nature (London)* **359**, 609–614.
7. Kingsley, D. M. (1994) *Trends Genet.* **10**, 16–21.
8. Dale, L., Howes, G., Price, B. M. J. & Smith, J. C. (1992) *Development (Cambridge, U.K.)* **115**, 573–585.
9. Jones, C. M., Lyons, K. M., LaPan, P. M., Wright, C. V. E. & Hogan, B. L. M. (1992) *Development (Cambridge, U.K.)* **115**, 639–647.
10. Nishimatsu, S., Suzuki, A., Shoda, A., Murakami, K. & Ueno, N. (1992) *Biochem. Biophys. Res. Commun.* **186**, 1487–1495.
11. Shoda, A., Murakami, K. & Ueno, N. (1994) *Biochem. Biophys. Res. Commun.* **198**, 1267–1274.
12. Hanks, S. K. & Quinn, A. M. (1991) *Methods Enzymol.* **200**, 38–62.
13. Mathews, L. S. & Vale, W. W. (1991) *Cell* **65**, 973–982.
14. Lin, H. Y., Wang, X.-F., Ng-Eaton, E., Weinberg, R. A. & Lodish, H. F. (1992) *Cell* **68**, 775–785.
15. Suzuki, A., Nagai, T., Nishimatsu, S., Sugino, H., Eto, Y., Shibai, H., Murakami, K. & Ueno, N. (1994) *Biochem. J.* **298**, 275–280.
16. Thies, R. S., Bauduy, M., Ashton, B. A., Kurtzberg, L., Wozney, J. M. & Rosen, V. (1992) *Endocrinology* **130**, 1318–1324.
17. Kaufman, R. J., Davies, M. V., Pathak, V. K. & Hershey, J. W. (1989) *Mol. Cell. Biol.* **9**, 946–958.
18. Frolik, C. A., Wakefield, L. M., Smith, D. M. & Sporn, M. B. (1984) *J. Biol. Chem.* **259**, 10995–11000.
19. Krieg, P. A. & Melton, D. A. (1984) *Nucleic Acids Res.* **12**, 7057–7070.
20. Moon, R. T. & Christian, J. L. (1989) *Technique* **1**, 76–89.
21. Nieuwkoop, P. D. & Faber, J., eds. (1967) *Normal Table of* Xenopus laevis (Daudine) (North-Holland, Amsterdam).
22. Harland, R. M. (1991) *Methods Cell Biol.* **36**, 685–695.
23. Kingsley, D. M. (1994) *Genes Dev.* **8**, 133–146.
24. Takuwa, Y., Ohse, C., Wang, E. A., Wozney, J. M. & Yamashita, K. (1991) *Biochem. Biophys. Res. Commun.* **174**, 96–101.
25. Suzuki, A., Nishimatsu, S., Shoda, A., Takebayashi, K., Murakami, K. & Ueno, N. (1993) *Biochem. J.* **291**, 413–417.
26. ten Dijke, P., Yamashita, H., Ichijo, H., Franzén, P., Laiho, M., Miyazono, K. & Heldin, C.-H. (1994) *Science* **264**, 101–104.
27. Suzuki, A., Shioda, N., Maeda, T., Tada, M. & Ueno, N. (1994) *Biochem. Biophys. Res. Commun.* **198**, 1063–1069.
28. Ebner, R., Chen, R.-H., Lawler, S., Zioncheck, T. & Derynck, R. (1993) *Science* **262**, 900–902.
29. Lawler, S., Candia, A. F., Ebner, R., Shum, L., Lopez, A. R., Moses, H. L., Wright, C. V. E. & Derynck, R. (1994) *Development (Cambridge, U.K.)* **120**, 165–175.
30. Estevez, M., Attisano, L., Wrana, J. L., Albert, P. S., Massagué, J. & Riddle, D. L. (1993) *Nature (London)* **365**, 644–649.
31. Brand, T., MacLellan, W. R. & Schneider, M. D. (1993) *J. Biol. Chem.* **268**, 11500–11503.
32. Kao, K. R. & Elinson, R. P. (1988) *Dev. Biol.* **127**, 64–77.
33. Cho, K. W. Y., Blumberg, B., Steinbeisser, H. & De Robertis, E. M. (1991) *Cell* **67**, 1111–1120.
34. Sato, S. M. & Sargent, T. D. (1991) *Development (Cambridge, U.K.)* **112**, 747–753.
35. Smith, J. C., Price, B. M. J., Green, J. B. A., Weigel, D. & Herrmann, B. G. (1991) *Cell* **67**, 79–87.
36. Moon, R. T. & Christian, J. L. (1992) *Cell* **71**, 709–712.
37. Smith, W. C., Knecht, A. K., Wu, M. & Harland, R. M. (1993) *Nature (London)* **361**, 547–549.