Bone morphogenetic proteins (BMPs) are members of the transforming growth factor (TGF)β superfamily of ligands that regulate many crucial aspects of embryonic development and organogenesis. Unlike other TGFβ ligands, co-receptors for BMP ligands have not been described. Here we show that DRAGON, a glycosylphosphatidylinositol-anchored member of the repulsive guidance molecule family, which is expressed early in the developing nervous system, enhances BMP but not TGFβ signaling. DRAGON binds directly to BMP2 and BMP4 but not to BMP7 or other TGFβ ligands. The enhancing action of DRAGON on BMP signaling is also reduced by administration of Noggin, a soluble BMP antagonist, indicating that the action of DRAGON is ligand-dependent. DRAGON associates directly with BMP type I (ALK2, ALK3, and ALK6) and type II (ActRII and ActRIIB) receptors, and its signaling is reduced by dominant negative Smad1 and ALK3 or -6 receptors. In the Xenopus embryo, DRAGON both reduces the threshold of the ability of Smad1 to induce mesodermal and endodermal markers and alters neuronal and neural crest patterning. The direct interaction of DRAGON with BMP ligands and receptors indicates that it is a BMP co-receptor that potentiates BMP signaling.

Transforming growth factor beta (TGFβ)1 superfamily ligands that include the TGFβ, bone morphogenetic protein (BMP), growth and differentiation factor, and nodal-related families play a pleiotropic role in vertebrate development by influencing cell specification, differentiation, proliferation, patterning, and migration (1, 2). These functions require the tight control of ligand production, ensuring a highly ordered spatiotemporal distribution and specific activation, via receptor complexes, of particular intracellular signaling pathways. The TGFβ/activin/nodal ligand subfamily contributes to the specification of endoderm and mesoderm in pregastrula embryos and at gastrula stages, to dorsal mesoderm formation and anterior-posterior patterning (3, 4). Later, TGFβ ligands influence the body axis and patterning of the nervous system (5). BMPs, a second major ligand subfamily, contribute to the ventralization of germ layers in the early embryo and suppress the default neural cell fate of the ectoderm (6). BMPs also participate later in development in the formation and patterning of the neural crest, heart, blood, kidney, limb, muscle, and skeletal system (7).

Signal transduction in the BMP subfamily is initiated by ligand binding to a receptor complex composed of two type I and two type II receptors. Three different BMP type I receptors (activin receptor-like kinase ALK2, ALK3, and ALK6) and three BMP type II receptors (BMP type II receptor (BMPRII), activin type IIA receptor (ActRIIA), activin type IIB receptor (ActRIIB)), each with intracellular serine/threonine kinase domains, have been identified (8). Ligand binding induces phosphorylation of the type I receptor by the type II receptor, which leads to phosphorylation of cytoplasmic receptor-activated Smads. The BMP subfamily signals through one set of receptor-activated Smads (Smad1, Smad5, and Smad8) whereas the TGFβ subfamily signals via another (Smad2, Smad3). The receptor-activated Smads form heteromeric complexes with a co-Smad, Smad4, which translocates from the cytoplasm to the nucleus to regulate gene expression.

Multiple modulators enhance or reduce TGFβ and BMP signaling. The access of TGFβ ligands to receptors is inhibited by the soluble proteins LAP, decorin, and α2-macroglobulin that bind and sequester the ligands (2). Soluble BMP antagonists include Noggin, chordin, chordin-like, the DAN/Cerberus protein family, and sclerostin (2). TGFβ ligand access to receptors is also controlled by membrane-bound receptors. BAMBI acts as a decoy receptor, competing with the type I receptor (9), β-glycan (TGFβ type III receptor) enhances TGFβ binding to the type II receptor (10–12), and endoglin enhances TGFβ binding to ALK1 in endothelial cells (13–15). Cripto, an EGF-CFC glycosylphosphatidylinositol (GPI)-anchored membrane protein, acts as a co-receptor, increasing the binding of the TGFβ ligands nodal, Vg1, and growth and differentiation factor 1 to activin receptors (16, 17) while blocking activin signaling.

Only co-receptors acting within the TGFβ/activin/nodal signal transduction pathway have been identified so far. We now find that DRAGON, a 436-amino-acid GPI-anchored protein identified by us and expressed early in the embryonic nervous system, enhances BMP signaling by forming a complex with the type I BMP receptors and the Smad2/3/5/8 coreceptor, DRAGON.
system (18), enhances BMP signaling in cells and developing embryos. DRAGON is a member of a gene family comprising two other GPI-linked proteins, repulsive guidance molecule (RGM) (19) and HFE2 (20) that have diverse roles. DRAGON produces homophilic and heterophilic cell-cell neuronal adhesion (18), RGM regulates retinotectal projections and neural tube closure (19, 21), while mutations in the human HFE2 locus are linked to juvenile hemochromatosis (20). Here, we show that DRAGON enhances BMP signaling, binds to specific BMP ligands, associates with BMP receptors, and potentiates BMP cellular signaling, indicating that DRAGON is a BMP co-receptor.

MATERIALS AND METHODS

DRAGON Polyclonal Antibody

The rabbit polyclonal DRAGON antibody was characterized and described previously (18). Briefly, a rabbit polyclonal antibody was raised against the peptide sequence TAAHASLEDAHPRKC (molecular weight, 2019.01), which is present in the C terminus of DRAGON upstream of its hydrophobic tail and affinity-purified using the same peptide. The antibody binds with high affinity to recombinant DRAGON expressed in HEK293T-transfected cells, recognizing a band of 50–55 kDa in Western blots. Western blots of protein extracts from neonatal and adult dorsal root ganglion and dorsal root ganglion primary cultures show a similar band with an additional lower band of 35–40 kDa, indicating possible proteolytic cleavage of endogenous DRAGON, similar to that found for chick DRG (19). Precubication of DRAGON antibody with 1 μM of the above antigenic peptide (4 h at room temperature) results in a loss of both bands by Western blot and staining signals by immunohistochemistry.

Immunohistochemistry

To obtain E2.5 embryos, 5-week-old female ICR mice were superovulated by injecting 5 IU of human chorionic gonadotropin (Sigma) 48 h after a prior administration of 5 IU of pregnant mare serum gonadotropin (Sigma). Treated females were mated with fertile male mice of the same strain and E2.5 embryos were flushed from oviducts. Embryos were washed with FBS containing 3% FBS (3% FBS-FBS) and fixed in 3.7% paraformaldehyde and 0.2% Triton X-100 for 10 min at room temperature. They were permeabilized for 30 min with 20 mM HEPES, 300 mM sucrose, 50 mM NaCl, 3 mM MgCl2, and 0.5% Triton X-100, pH 7.4. After blocking with 3% FBS-FBS for 30 min, embryos were incubated with the DRAGON antibody diluted 1:2000 in 3% FBS-FBS overnight at 4 °C, washed, and then incubated with fluorescein isothiocyanate-conjugated anti-rabbit IgG (Jackson Immunological Research Laboratories) for 2 h at room temperature. After washing with 3% FBS-FBS for 30 min at room temperature, embryos were mounted with mounting medium containing propidium iodide (Vector Laboratories, Inc.).

Whole mount immunohistochemistry in mouse E10.5 embryos was carried out as described previously (22). Briefly, freshly dissected embryos were fixed with 4% paraformaldehyde overnight at 4 °C, washed in saline for 2 h, and then soaked in 80% methanol series. Endogenous peroxidase activity was quenched with 3% H2O2 in 80% methanol and saline for 2 h, and then soaked in 80% methanol series. Cell lysates were harvested 48 h post-transfection, and luciferase activity was monitored by a plate reader luminometer. Relative light units were determined and corrected for transfection efficiency using β-galactosidase activity, as all plates were co-transfected with β-galactosidase expression construct to normalize transfection efficiency.

Ligands

Carrier-free human BMP2, -4, and -7, as well as TGFβ1, -2, and activin were purchased from R&D systems (Minneapolis, MN). Noggin was also purchased from R&D systems.

Affinity Labeling, Immunoprecipitation, and Immunoblotting

TGFβ superfamily receptor constructs (28) were transiently transfected into COS-1 cells with Lipofectamine (Invitrogen). The next day cells were washed with medium containing 0.2% FBS for 10 min and treated overnight with BMP2, BMP4, or TGFβ3, as described previously (18). Cell lysates were harvested 48 h post-transfection, and luciferase activity was monitored by a plate reader luminometer. Relative light units were determined and corrected for transfection efficiency using β-galactosidase activity, as all plates were co-transfected with β-galactosidase expression construct to normalize transfection efficiency.

RESULTS

DRAGON Expression

DRAGON is expressed in mouse E2.5 pre-implantation embryos (Fig. 1A), and in postimplantation embryos (>E7) (Fig. 1B). In E10.5 embryos, DRAGON is found along the neural tube, in the dorsal root ganglia, and in the tips of the neural folds and the tail bud (Fig. 1C) as assessed by whole mount immunohistochemistry. Xenopus orthologs of both DRAGON and RGM are expressed maternally and throughout development and are detected at high levels by tadpole stages (Fig. 1D). Analysis by whole mount in situ hybridization of RGM expression at stage 12 reveals high levels in the ectoderm by the blastopore (Fig. 1E, i). More restricted expression is seen by stage 17 and stage 20 in the neural plate, specifically in the dorsal aspect of the neural tube (Fig. 1E, ii and iii). By stage 35, the expression of RGM is detected in the hindbrain, midbrain,
and forebrain regions, as well as the branchial arches. In addition, expression is seen posteriorly in the somites and tail bud (Fig. 1E, iv).

The expression pattern of DRAGON and RGM at different embryonic stages in both the developing mouse and *Xenopus* is comparable with the expression of the BMP type I receptors, ALK3 and ALK6 and BMP type II receptor (31, 32) (Fig. 1). This observation prompted us to investigate whether DRAGON contributes to or modulates TGFβ/H9252 superfamily signal transduction.

**DRAGON Enhances BMP but Not TGFβ**

Intracellular Signaling

TGFβ superfAMILY ligands are classified as TGFβ or BMP-like based on the particular Smad-dependent intracellular pathways they activate. We examined whether DRAGON activates these pathways using specific BMP- and TGFβ-responsive luciferase reporters in LLC-PK1 (kidney epithelial cells), as these cells are highly responsive to many TGFβ family members and thereby allow a parallel analysis of any action of DRAGON on TGFβ superfAMILY signaling.

To determine whether DRAGON alters BMP signaling we used the BMP-inducible promoter BRE-Luc, an Id1 promoter-derived reporter construct (24). BMP2 stimulates BRE-Luc activity in LLC-PK1 cells (Fig. 2). Co-expression of DRAGON with BRE-Luc in the absence of exogenous ligand increases BRE-Luc activity to levels comparable with that achieved by BMP2 stimulation in the absence of DRAGON (Fig. 2A). To assess whether DRAGON regulates the signaling produced by BMP ligands, LLC-PK1 cells were co-transfected with the BRE-Luc reporter and DRAGON (2 and 20 ng) and incubated with BMP2. BMP2 (50 ng/ml) induces a 6-fold increase in luciferase activity in the absence of DRAGON and a 14-fold increase in DRAGON co-transfected cells (Fig. 2A). DRAGON expression had no effect on TGFβ signaling, assessed by using a TGFβ-responsive promoter (TGFβ-responsive CAGA reporter (12)) in LLC-PK1 cells (data not shown).

**FIG. 1.** **DRAGON is expressed during embryogenesis.**

A, DRAGON expression in mouse pre-implantation embryos (E2.5) assessed with a DRAGON antibody and visualized by fluorescein isothiocyanate green fluorescence. Nuclei of the embryonic cells are visualized by CY3 red fluorescence (propidium iodide). Pre-adsorption of the DRAGON antibody with the peptide antigen (Pept.) was used as negative control. B, Northern blot showing DRAGON mRNA expression in mouse embryos at embryonic stages E7–17. Cyclophilin mRNA levels were used as loading controls. C, immunohistochemical study of the expression and distribution of DRAGON in mouse E10.5 embryos. D, expression of *Xenopus* DRAGON (RGMb) and RGMa assessed by reverse transcription (RT)-PCR at the indicated developmental stages. Orthidine decarboxylase (ODC) levels were used as internal control. E, analysis by whole mount in situ hybridization of expression of *Xenopus* RGMa at stages 12 (i), 17 (ii), 20 (iii), and 35 (iv). Transverse sections through stained stage 12 (top right) and stage 20 (bottom left) embryos are also represented. bp, blastopore; post, posterior; ant, anterior; nc, notochord; rp, roof plate; hb, hindbrain; mb, midbrain; fb, forebrain; ba, branchial arches.
The activity was assessed after incubation in the absence (white bars) or presence (black bars) of BMP2 (1 nM) in the culture medium. Luciferase activity is represented as relative light units (RLU). DRAGON increases and potentiates BMP2-mediated signaling. LLC-PK1 cells were transiently transfected with BRE-Luc in the presence or absence of DRAGON and treated with increasing amounts of Noggin as indicated. As a positive control, BRE-Luc-transfected LLC-PK1 cells were treated with BMP2 (1 nM) in the presence of Noggin (50 ng/ml). As a negative control, LLC-PK1 cells transfected with the TGFβ-responsive reporter construct MLP-Luc were treated with TGFβ (1 nM) in the presence of Noggin (50 ng/ml). C, 10 T1/2 cells transiently transfected with the BMP-responsive I-BRE-Luc reporter and DRAGON were incubated with increasing doses of BMP2 (25–500 pM).

DRAGON significantly enhances BMP2-mediated reporter activity in this cell line at BMP2 concentrations of 25–300 pM (Fig. 2A). This effect is lost at 300 pM or higher, where maximal signaling is achieved.

Similar results were obtained using LLC-PK1, 10 T1/2, and HepG2 cell lines and different BMP-responsive promoters (I-BRE-Luc, BRE-Luc, and Max2-Luc) (Fig. 2, and data not shown). DRAGON significantly increases cellular sensitivity, therefore, to low doses of BMP2 in a manner that is neither reporter- nor cell-specific. These results suggest that the regulatory role of DRAGON in BMP signaling is a generalized phenomenon. This finding prompted us to study the mechanism of the enhancement of BMP signaling by DRAGON and where and how it interacts with BMP signaling components.

Mechanism of the Enhancement of BMP Signaling by DRAGON

DRAGON could potentially enhance BMP signaling through a direct modulation of the BMP signaling complex or through an independent receptor pathway that converges on the intracellular Smads. We therefore investigated whether DRAGON binds to BMP ligands, interacts directly with BMP receptors, and whether a dominant negative BMP receptor or Smad mutants reduce the DRAGON-induced enhancement of BMP signaling.

DRAGON Binds to BMP but Not TGFβ Ligands—To determine whether cell surface-localized DRAGON binds directly to BMP ligands, COS-1 cells expressing DRAGON were incubated with [125I]-BMP2 or [125I]-TGFβ and receptor-bound ligand cross-linked. HA-tagged BMP type I receptor (ALK6) or TGFβ type II receptor (TβRII) served as positive controls for BMP2 or TGFβ binding, respectively. Immunoprecipitation of DRAGON from [125I]-BMP2- but not [125I]-TGFβ-labeled cells revealed the presence of two [125I]-BMP2-bound proteins of ~55–65 kDa (Fig. 3A), consistent with the expected size of a BMP-bound DRAGON. The presence of two bands suggests that cell surface-localized DRAGON may be subject to post-translational modification. These data, indicating that cell surface-expressed DRAGON can bind BMP2 but not TGFβ, prompted us to assess whether DRAGON can directly bind BMP ligands.

We examined whether DRAGON directly binds to BMP ligands using a soluble DRAGON-Fc fusion protein in a cell-free binding system (12). DRAGON-Fc binds [125I]-BMP2 with high affinity and an apparent dissociation rate constant (Kd) of 1.5 nM (Fig. 3B). This binding is competed with an excess of unlabeled BMP2 as well as by BMP4 (4 nM) (Fig. 3, B and C). In contrast, BMP7 (4 nM), activin A, TGFβ1, -2, or -3 (4 nM each) do not competitively inhibit the binding of DRAGON-Fc to BMP2 (Fig. 3C). Pretreatment of cells with DRAGON-Fc decreases BMP2- but not BMP7-mediated activation of the BRE-Luc promoter in a dose-dependent manner (60 and 300 ng/ml) (Fig. 3D) but had no effect on TGF-β1-dependent activation of
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the CAGA-Luc promoter (Fig. 3E). DRAGON directly interacts with members of the BMP but not the TGFβ ligand family, with a preference for BMP2 and BMP4.

**DRAGON Enhances BMP Signaling Only when Expressed on the Cell Surface**—To assess whether the GPI anchor is required for the action of DRAGON on BMP signaling, we used the DRAGON-Fc fusion protein where the C-terminal GPI anchor is deleted and replaced by human Fc (18). When cotransfected with the BRE-Luc reporter construct this DRAGON-Fc, which is secreted into the medium, fails to increase BMP signaling (Fig. 4A). Western blot confirms the expression of both DRAGON and DRAGON-Fc in the transfected cells (Fig. 4B).

These results demonstrate that the expression of DRAGON on the cell surface is required for its action on BMP signaling.

**DRAGON Binds to BMP Type I and Type II Receptors**—BMP type I and type II receptors are essential for mediating BMP responses. To test whether DRAGON directly interacts with BMP type I and type II receptors, HEK293T cells were transiently transfected either with DRAGON alone or with HA epitope-tagged type I and type II BMP receptors. Anti-Dragon immunoprecipitation followed by anti-HA immunoblotting reveals that DRAGON associates directly with all BMP type I receptors, (ALK2, ALK3, and ALK6) (Fig. 5A) and with the BMP type II receptors ActRII and ActRIIB (Fig. 5B).

**DRAGON Signaling Is Blocked by Dominant Negative ALK3, -6, and Smad1**—We used dominant negative type I receptors (ALK1-DN, ALK3-DN, and ALK6-DN), which are deficient in kinase activity and unable to phosphorylate Smads (33), to confirm that DRAGON acts through modulation of BMP receptors and subsequent activation of the downstream Smad1 pathway (Fig. 5, C and D). Co-expression of DRAGON with ALK3-DN and ALK6-DN in LLC-PK1 cells decreases DRAGON-mediated induction of I-BRE luciferase activity to baseline, whereas co-expression of ALK1-DN does not affect DRAGON-mediated BMP signaling (Fig. 5C). Co-expression of DRAGON in LLC-PK1 cells with a Smad1 dominant negative mutant that lacks the C-terminal phosphoacceptor domain (15, 34) also dose dependently reduces DRAGON-induced signaling. In contrast, co-expression with wild type Smad1 enhances this signaling (Fig. 5D). These studies demonstrate that

**FIG. 3.** DRAGON interacts directly with BMP2 and BMP4. A, DRAGON expressed in COS cells binds to BMP2 but not TGFβ. COS-1 cells were transiently transfected with DRAGON (DR), HA-tagged ALK6 (A6/HA), or TglyR (RII/HA). Cells were affinity-labeled with [125I]-BMP2 (left panel) or [125I]-TGFβ (right panel) and lysates subjected to immunoprecipitation (IP) or immunoblotting with the indicated antibodies. [125I]-Labeled ligand binding to receptors was detected by phosphorimaging. B, DRAGON-Fc binds directly to BMP2. [125I]-BMP2 was incubated with increasing amounts (10–50 ng) of purified DRAGON-Fc then added to protein A-coated plates, and bound radioactivity was measured (left graph). 20 ng of DRAGON-Fc were incubated with [125I]-BMP2 in the presence of increasing amounts of competing unlabeled BMP2 (0–42 nm) (right graph). C, DRAGON-Fc binds directly to BMP2 and BMP4 but not BMP7 or TGFβ ligands. DRAGON-Fc was incubated with [125I]-BMP2 in the presence of unlabeled BMP2, BMP4, BMP7, Activin A (ActA), TGFβ1, TGFβ2, and TGFβ3, and [125I]-BMP2 binding was measured. D, DRAGON-Fc reduces BMP2 mediated signaling. LLC-PK1 cells were transiently transfected with BRE-Luc construct and treated with BMP2 (150 ng/ml) and DRAGON-Fc (60 and 300 ng/ml). E, DRAGON-Fc (60 ng/ml) reduces BMP2 but not BMP7- or TGFβ1 (1 nm each)-mediated signaling. LLC-PK1 cells were transiently transfected with BRE-Luc and treated with BMP2 or BMP7 or transiently transfected with 3TP-Lux construct and treated with TGFβ1 in the absence or presence of DRAGON-Fc.
DRAGON exerts its effect on BMP signaling via BMP receptors and potentiates Smad1 signaling.

**DRAGON Enhances BMP Signaling in Xenopus Embryos**

BMP ligands play pivotal roles in the gastrulation of the embryo (35) and regulate the formation of the mesoderm and endoderm (36). An analysis of mice lacking genes encoding BMPs, BMP receptors, and their downstream signal transducers reveals major early and late embryonic developmental alterations, with defects in mesoderm and endoderm formation (37). We have examined whether DRAGON expression in Xenopus embryos enhances BMP signaling and synergizes with BMP signal transduction components to facilitate BMP signaling in vivo. Mouse DRAGON mRNA was injected alone or in combination with Smad1 mRNA in Xenopus embryos at the two-cell stage. We then analyzed the expression levels of mesodermal or endodermal tissue markers as indicators of activation of BMP signaling. DRAGON co-injection with Smad1 leads to the induction of mRNA for the pan-mesodermal marker Xbra and two endodermal markers, mix1 and mixer in a dose-dependent manner (Fig. 6A). Injected alone, neither DRAGON nor Smad1 at the low amounts administered induce the expression of the mesodermal and endodermal markers (Fig. 6A). The reduction in the threshold of the activity of Smad1 by DRAGON indicates the enhancement of BMP signaling in the embryo and is consistent with the results seen in mammalian cells (Fig. 2).

**DRAGON Regulates Neural Patterning in Xenopus Embryos**

Genetic experiments have suggested that various BMPs can promote dorsal neural cell fates (38) and act as morphogens to specify dorsal neural cell types (39). However, the contribution of the different BMP signaling components to neural patterning is not fully elucidated. To determine the effect of DRAGON expression on tissue patterning, we analyzed fate changes in Xenopus embryonic explants by reverse transcription-PCR for a variety of molecular markers. DRAGON mRNA was injected at the two-cell stage in the animal caps, and cultured ectodermal explants were analyzed for changes in gene expression. DRAGON induces expression of the anterior pan-neural marker Nrp1, the cement gland marker (the most anterior structure in the tadpole) XAG, as well as the early heart marker nkx2.5 (Fig. 6B). Up-regulation of both neural and heart markers by DRAGON (Fig. 6B) suggests that the induction of neural tissue by DRAGON is not direct but occurs via formation of dorsal mesoderm, which induces neural tissue.
To determine whether DRAGON expression alters neural patterning, we injected mouse DRAGON mRNA into one cell at the two-cell stage in the animal pole of the Xenopus embryo and left the injected embryos to develop until early tadpole stages (Fig. 6C). The uninjected cell serves as control for the DRAGON-injected experimental side in the same embryo. Overexpression of DRAGON results in an increase in N-tubulin (a general neuronal differentiation marker) expression, as shown by the appearance of ectopic patches of N-tubulin-expressing cells (Fig. 6C) and a decrease in neural crest derivatives, as shown by a reduction in twist (an anterior neural crest marker) expression on the injected side (Fig. 6C) (40). DRAGON may contribute, therefore, to the patterning of the developing nervous system by promoting a neuronal phenotype and inhibiting neural crest differentiation.

**DISCUSSION**

DRAGON was identified by us in a genomic-binding screen for promoter regions of effector genes regulated by the developmentally regulated paired homeodomain transcription factor, DRG11 and is a 436-amino acid member of the RGM family of GPI-anchored proteins (18). We show here that DRAGON binds to BMP ligands and receptors, and enhances BMP signaling, and conclude that it is a BMP co-receptor.

Given the wide spectrum of responses to the TGFβ superfamily, mechanisms for positive and negative modulation of their signaling exist to exert tight spatiotemporal regulation. Positive regulation amplifies signals by reducing activation threshold and potentiating biological activity, whereas negative regulation limits the magnitude of signals or terminates signaling. Regulation occurs at the extracellular, membrane, cytoplasmic, and nuclear levels and contributes to the formation and response to gradients of morphogens in development. The role of DRAGON in the embryo may be to differentially increase or boost the sensitivity of cells to low levels of BMP ligand. Such low levels will occur within the spatial BMP gradients established in the embryo, and DRAGON expression may enable cells to respond earlier or with a greater response to a particular level of BMP, than those that do not express DRAGON. Therefore, a differential sensitivity to BMP ligands produced by DRAGON expression may contribute to tissue patterning.

DRAGON interacts directly with specific BMP ligands (BMP2 and -4 but not BMP7) in the absence and presence of BMP type I and type II receptors. How DRAGON enhances signaling by the receptor complex needs to be determined. Does it change the affinity of BMP receptor-ligand interactions or facilitate the formation of heteromeric receptor complexes between type I and type II receptors? As a GPI-anchored protein with no transmembrane domain, DRAGON is unlikely to directly alter either the intracellular kinase activity of the BMP receptors or their coupling to the receptor-activated Smads, but it may stabilize the receptor complex. The action of DRAGON, like other GPI-anchored proteins, may depend on localization in lipid rafts (41) and in this way contribute to the assembly and trafficking of the receptor complex in microdomains.

DRAGON is not necessary for BMP2 and -4 signaling but rather enhances signaling in response to low concentrations of these BMP ligands. In this respect, DRAGON differs from nodal, Vg1, and growth and differentiation factor 1, which require a co-receptor from the epidermal growth factor-Cripto-
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FRL1-Cryptic protein family to generate signals (15). Although DRAGON transfection is sufficient, in the absence of exogenous ligand to increase BMP signaling, these actions are BMP ligand-dependent in that they are terminated by administration of Noggin, which sequesters BMP but not TGFβ ligands.

The phenotypes of mice with null mutations of genes encoding BMPs, BMP receptors, and their downstream signal transducers reveal the contribution of BMP signaling to early developmental processes such as gastrulation and formation of mesodermal and endodermal tissues, left-right asymmetry, neural patterning, and organogenesis (37). That overexpression of DRAGON in Xenopus embryos increases expression of mesodermal and endodermal tissue markers and has a differential action on neuronal and neural crest pathways is compatible with a role for DRAGON in the patterning of the nervous system. Given the maternal expression of DRAGON, presence in pre-implantation embryos, and highly organized and dynamic expression in the developing nervous system, DRAGON may have multiple roles in the embryo, potentiating BMP signaling at specific times and locations.

DRAGON and the other two members of the RGM family are highly conserved across species in both vertebrate and invertebrates. Mouse, human, Xenopus, chick, Zebrafish, Fugu, and Caenorhabditis elegans orthologs have been identified (18). The other two members of the RGM GPI-anchored family, RGMa and DRAGON-like muscle (RGMc/HFE2), also enhance BMP signaling (data not shown). RGMa and DRAGON/RGMb are both expressed at high levels in the developing and adult nervous systems, whereas DRAGON-like muscle (RGMc/HFE2) is found almost exclusively in skeletal and cardiac muscle and liver (18, 20, 21). Although RGMa has a repulsive guidance role in the retina (21). DRAGON is the first identified co-receptor for the BMP signaling pathway and binds to BMP ligands and receptors to enhance BMP signaling. DRAGON overexpression produces an ectopic neurons and decreases neural crest cells in Xenopus embryos indicating that DRAGON, by virtue of its facilitating or enhancing role in BMP signaling, is likely to regulate the response of developing neurons to BMP ligand gradients and thereby influence patterning and differentiation in the embryo.

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