BMP Type II Receptor Is Required for Gastrulation and Early Development of Mouse Embryos

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Bone morphogenetic proteins (BMPs), members of the transforming growth factor-β superfamily, play a variety of roles during mouse development. BMP type II receptor (BMPR-II) is a type II serine/threonine kinase receptor, which transduces signals for BMPs through heteromeric complexes with type I receptors, including activin receptor-like kinase 2 (ALK2), ALK3/BMPR-IA, and ALK6/BMPR-IB. To elucidate the function of BMPR-II in mammalian development, we generated BMPR-II mutant mice by gene targeting. Homozygous mutant embryos were arrested at the egg cylinder stage and could not be recovered at 9.5 days postcoitum. Histological analysis revealed that homozygous mutant embryos failed to form organized structure and lacked mesoderm. The BMPR-II mutant embryos are morphologically very similar to the ALK3/BMPR-IA mutant embryos, suggesting that BMPR-II is important for transducing BMP signals during early mouse development. Moreover, the epiblast of the BMPR-II mutant embryo exhibited an undifferentiated character, although the expression of tissue-specific genes for the visceral endoderm was essentially normal. Our results suggest that the function of BMPR-II is essential for epiblast differentiation and mesoderm induction during early mouse development.

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Key Words: bone morphogenetic protein; type II serine/threonine kinase receptor; gene targeting; mouse embryo; gastrulation; anterior-posterior axis.

INTRODUCTION

Bone morphogenetic proteins (BMPs) constitute a large group of secreted signaling molecules of the transforming growth factor-β (TGF-β) superfamily and exert multifunctional effects on various cell types including osteoblasts, chondroblasts, neural cells, and epithelial cells (reviewed in Hogan, 1996; Kawabata et al., 1998; Sakou, 1998). BMPs function via binding to two types of serine/threonine kinase receptors, type I and type II receptors, that are both necessary for signaling (reviewed in Kawabata et al., 1998; Massagué, 1998). Three different type II receptors, i.e., BMP type II receptor (BMPR-II) and activin type II receptors (ActR-II and ActR-IIB), bind BMPs (Rosenzweig et al., 1995; Liu et al., 1995; Nohno et al., 1995; Yamashita et al., 1995), and three type I receptors, i.e., activin receptor-like kinase (ALK) 3/BMPR-IA, ALK6/BMPR-IB, and ALK2, serve as BMP type I receptors (Koenig et al., 1994; ten Dijke et al., 1994a,b; Liu et al., 1995; Macías-Silva et al., 1998; Ebisawa et al., 1999; Fujii et al., 1999). Upon ligand stimulation, the type II and type I receptors for BMPs phosphorylate intracellular substrates, i.e., Smad1, Smad5, and Smad8, which then form complexes with Smad4, translocate into the...
nucleus, and regulate the transcription of various target genes including Tlx-2 (reviewed in Heldin et al., 1997).

BMPs play critical roles in the establishment of the basic embryonic body plan. Several studies in Xenopus embryos have revealed that BMPs, a member of the BMP family, induces ventral mesoderm, whereas activins induces dorsal mesoderm (reviewed in Smith, 1995; Harland and Gerhart, 1997). Overexpression of dominant-negative forms of BMP receptors, including ALK3/PR-IA and BMP-RII, leads to abnormal mesoderm formation and patterning (Graff et al., 1994; Suzuki et al., 1994; Ishikawa et al., 1995). Moreover, overexpression of Smad1 (Graff et al., 1996; Thomsen, 1996) and Smad5 (Suzuki et al., 1997) produces patterning defects that are identical to those observed following misexpression of BMP4, i.e., ventralization and repression of neural fate in Xenopus embryos.

Studies by gene targeting have demonstrated that BMP signals are required for gastrulation of mouse embryos. The majority of the homozygous null BMP4 embryos die at or around the time of gastrulation without forming embryonic mesoderm (Winnier et al., 1995; Lawson et al., 1999). BMP2 mutant embryos form the mesodermal cells, but fail to close the proamniotic canal and also exhibit a defect in cardiac development (Zhang et al., 1996). Among the type I receptors for BMPs, ALK3/PR-IA (Bmpr) mutant embryos fail to form organized structure and mesoderm (Mishina et al., 1995). The ALK2 (ActRIIA<sup>−/−</sup>) mutant embryos are also arrested at the early gastrulation stage, displaying disruption of mesoderm formation (Gu et al., 1999; Mishina et al., 1999). The phenotypes of the ALK3 mutant embryos appear to be more severe than those of the ALK2 mutant embryos.

Analyses by gene targeting have revealed the critical functions of Smad4 and Smad5, which act downstream of BMP receptors, during embryonic development. Smad4 mutant embryos die before 7.5 days postcoitum (E7.5) with gastrulation defect and abnormal visceral endoderm (Sirard et al., 1998; Yang et al., 1998). Smad5 mutant embryos die between E9.5 and E11.5 and exhibit multiple defects including lack of normal vasculature (Yang et al., 1999; Chang et al., 1999). Tlx-2 is a homeobox gene expressed in the primitive streak of mouse embryos and functions as a downstream target of BMP signaling. The Tlx-2 mutant embryos display severe defects in primitive streak and mesoderm formation (Tang et al., 1998).

Activin type II receptors, ActR-II and ActR-IIB, have been shown to bind osteogenic protein-1 (BMP-7), BMP-2, BMP-6, and growth/differentiation factor-5 as well as activins (Ya-mashita et al., 1995; Hoodless et al., 1996; Nishitoh et al., 1996; Ebisawa et al., 1999). In embryos from E5.5 to E7.5, the strong expression of ActR-IIB is observed in the embryonic ectoderm and weak expression in the extraembryonic ectoderm, whereas ActR-II is present at a lower level than ActR-IIB in E6.5 embryos (Manova et al., 1995; see below). While embryos lacking either ActR-II or ActR-IIB develop to term with no gross defects in mesoderm formation (Matzuk et al., 1995; Oh et al., 1997), embryos lacking both receptors are arrested at the egg cylinder stage before gastrulation (Song et al., 1999).

To determine the function of BMPR-II in mammalian development, we disrupted the mouse BMPR-II gene by homologous recombination. BMPR-II mutant embryos were developmentally arrested at egg cylinder stage and failed to form organized structure. Our observations suggest that BMPR-II is essential for BMP signaling pathway in early mouse development.

**MATERIALS AND METHODS**

**Construction of the Targeting Vector**

A 129/Sv mouse genomic library (Stratagene; Lambda Fix II) was screened with the 3.1-kb human BMPR-II cDNA (Kawabata et al., 1995), and the genomic organization was determined previously (Beppu et al., 1997). The No. 8 lambda clone, which contains exons 4, 5, and 6, was used to construct a replacement gene targeting vector. A 2.4-kb Cia1−Scal fragment containing exons 4 and 5 was deleted and replaced by a cassette of SA IRES/LacZ/loxP/pgk neo/loxP (Saitou et al., 1998). In this cassette, the pgk neo sequence was in the orientation opposite to the other sequences. The targeting vector contains a 7.0-kb 5′ homology region (NotI−ClaI fragment, NotI site from a linker of lambda phage vector) and a 2.0-kb 3′ homology region (Scal−EcoRI fragment). A coding region of the dipheria toxin A gene (Yagi et al., 1990) was attached to the 3′ end for negative selection.

**Electroporation and Selection of ES Cells**

The J1 ES cells (Li et al., 1992) were transfected with a linearized targeting vector by electroporation and selected in G418-containing medium as described (Saitou et al., 1998). The genotypes of G418-resistant clones were analyzed by Southern blot hybridization using a 0.7-kb EcoRI-PstI genomic fragment as a 3′ external probe. Four positive recombinant clones were obtained and checked for single integration by hybridization with a neo probe.

**Generation of Chimeric Mice and Germ-Line Transmission of the BMPR-II Mutant Allele**

Two of the BMPR-II mutant ES clones were microinjected into blastocysts derived from C57BL/6J mice. The resulting chimera were bred to C57BL/6J females, and F1 agouti offspring were analyzed for the presence of the BMPR-II mutant allele by Southern blotting. The chimeras from one cell line, No. 291, transmitted the mutation through the germ line. F1 mice heterozygous for the mutation were intercrossed to generate F2 offspring.

**Genotype Analysis**

Genotypes were determined by Southern blotting or PCR. For PCR analysis, three primers were used in each reaction. The mutant allele was amplified using primers A and C. This primer pair amplifies a 260-bp fragment from the mutant BMPR-II allele. A 200-bp fragment from the wild-type allele was amplified using
sequences of the primers are as follows: primer A, 5'-GCTAA AGCGC ATGCT CCAGA CTGCC TTG-3'; primer B, 5'-TCACA GCATG AACAT GATGG AGGCG G-3'; primer C, 5'-AGGTT GCCCT GGAAC CTGAG GAAAT C-3'.

Histology and Whole-Mount RNA in Situ Hybridization

For histological analysis, embryos in utero were fixed in 4% paraformaldehyde/PBS(−) at 4°C overnight, dehydrated, and embedded in paraffin. Sagittal sections (5 μm thick) were cut and stained with hematoxylin and eosin.

Whole-mount in situ hybridization was performed basically as described (Wilkinson, 1992). The following digoxigenin-labeled antisense RNA probes were used for whole-mount in situ hybridization studies: mouse BMPR-II (Beppu et al., 1997), ActR-II (Mathews et al., 1991), ActR-IIB (Attisano et al., 1992), Brachyury(T) (Wilkinson et al., 1990), and hepatocyte nuclear factor (HNF) 3β (Sasaki et al., 1993). The DNA fragment of Cripto (Ding et al., 1998) was cloned by RT-PCR from total RNA of C57BL/6J embryos at E6.5 and inserted into pGEM-T vector (Promega) to use as probe.

Expression of Tissue-Specific Genes for Visceral Endoderm

Total RNA was extracted from E7.5 normal and mutant embryos and decidual tissue using Isogen (NipponGene). For each embryo, 0.1 μg of total RNA was reverse transcribed using MMLV-RT (Gibco BRL) with random hexamer primers. The expression patterns of GATA4, HNF1, HNF4, α-fetoprotein (AFP), and transferrin (TFN) in normal and mutant embryos were compared by semi-quantitative RT-PCR analysis. The PCR amplification of the cDNA remained linear after 30 cycles (data not shown). The PCR products were analyzed by Southern blot using end-labeled internal primers as probes. Primers used for PCR amplification and internal probing are described (Duncan et al., 1997; Sirard et al., 1998).

RESULTS

Expression of BMPR-II in Early Mouse Embryos

We first analyzed the expression pattern of BMPR-II at E6.5–7.5 by whole-mount in situ hybridization and compared it with that of other type II receptors for BMPs, i.e., ActR-II and ActR-IIB. The sequences of open reading frames

FIG. 1. Expression of type II receptors for BMPs during gastrulation analyzed by whole-mount in situ hybridization. (A and B) Expression patterns of BMPR-II at E6.5 (A) and E7.5 (B). The embryos are hybridized with antisense probe (left) and sense probe (right). BMPR-II transcripts were ubiquitously distributed between E6.5 and E7.5. (C–F) Expression patterns of ActR-II (C, D) and ActR-IIB (E, F) in embryos at E6.5 (C, E) and E7.5 (D, F). Bar, 200 μm.
of these type II receptors were used as antisense RNA probes. We found that all three type II receptors were expressed at E6.5. The expression levels of BMPR-II and ActR-IIB transcripts were higher than that of ActR-II (Figs. 1A, 1C, and 1E). BMPR-II was expressed uniformly in embryonic and extraembryonic regions, whereas ActR-II and ActR-IIB were expressed at higher levels in the epiblast. Similar patterns of BMPR-II, ActR-II, and ActR-IIB expression were observed in E7.5 embryos (Figs. 1B, 1D, and 1F).

**Disruption of the BMPR-II Gene Results in Early Embryonic Lethality**

To mutate the BMPR-II gene in mouse embryonic stem (ES) cells, a targeting vector for replacement of exons 4 and 5, which encode the entire transmembrane domain and the amino-terminal region of the kinase domain, was constructed as shown in Fig. 2A. The linearized targeting vector was transfected into ES cells by electroporation. Of 77 G418-resistant colonies screened by Southern blot analysis and primers A, B, and C for genotyping by PCR are indicated as bars and arrowheads, respectively. X, XbaI; N, NotI; C, ClaI; RI, EcoRI; RV, EcoRV; S, SacI. A coding region of the diphtheria toxin A (MC1DT-A) was attached to the 3' end for negative selection. (B) Southern blot analysis of genomic DNA isolated from targeted ES clones digested with XbaI for the 3' external probe and EcoRV for neo probe. A random-integrated clone is shown as negative control. (C) Southern blot analysis of tail DNAs from offspring generated by intercrosses of F1 heterozygotes. No liveborn homozygous mutants were recovered. (D) PCR analysis of genotypes of E7.5 embryos generated by intercrosses of F1 heterozygotes.
TABLE 1
Phenotype and Genotype Analyses of Offspring from Intercrosses of BMPR-II Heterozygous Mice

<table>
<thead>
<tr>
<th>Age</th>
<th>Normal</th>
<th>Abnormal</th>
<th>Resorption</th>
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<td>E3.5</td>
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<td>0</td>
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<tr>
<td>E6.5</td>
<td>18</td>
<td>4</td>
<td>0</td>
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<tr>
<td>E7.5</td>
<td>35</td>
<td>20</td>
<td>0</td>
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<tr>
<td>E8.5</td>
<td>23</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>E9.5</td>
<td>16</td>
<td>0</td>
<td>4</td>
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<tr>
<td>Postnatal</td>
<td>61</td>
<td>0</td>
<td>0</td>
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<td>++/+</td>
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<td>14</td>
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Wild type, 70.5% were heterozygous, and none were homozygous (Fig. 2C; Table 1), indicating that BMPR-II mutant mice died during embryogenesis.

To determine when mutant embryos die in utero, embryos from heterozygous intercrosses were dissected and examined at different days of gestation (Table 1). The BMPR-II homozygous mutant embryos were recovered at a normal Mendelian ratio at E6.5–8.5, but all the mutant embryos were abnormal. At E6.5–8.5, 76 (73.1%) of 104 embryos were normal and 28 (26.9%) embryos were abnormal. Genotyping by PCR revealed that the normal embryos were either wild type or heterozygous for the BMPR-II mutation, while all the abnormal embryos were BMPR-II homozygous mutants (Fig. 2D; Table 1). The normal Mendelian ratio at E6.5–8.5, but before gastrulation its transcript becomes restricted to the region of the proximal rim of the epiblast (Fig. 3A and 3B). At E8.5, the disorganized mutant embryos did not exhibit increase in size compared with the E7.5 mutant embryos (Figs. 3E and 3F). Four of 20 decidua contained resorption sites and no homozygous mutants were recovered at E9.5. These results suggest that development of BMPR-II mutant embryos was arrested prior to gastrulation.

Morphological Defects in the BMPR-II Mutant Embryos

To determine the defects in the BMPR-II mutant embryos, histological analysis of normal embryos and mutant embryos at E6.5 and E7.5 was performed. At E6.5, normal littermates were well elongated with clear morphological distinction between the embryonic and the extraembryonic portions of the embryos, whereas the homozygous mutant embryos were disorganized and considerably smaller than their normal littermates (Figs. 3G and 3H). At E7.5, the growth of the mutant embryos was further retarded. Unlike the wild-type embryos, in which the mesoderm layer, amnion, and chorion were formed, the mutant embryos showed defects similar to those observed at E6.5 and contained no mesoderm (Figs. 3I and 3J). A small proamniotic cavity was present in the mutant embryos, indicating that initial cavity formation had occurred (Fig. 3J). These results indicate that BMPR-II mutant embryos are arrested at the egg cylinder stage before gastrulation.

To further characterize the gastrulation defects, we examined the expression of mesodermal marker genes. E7.5 embryos were examined by whole-mount in situ hybridization for expression of Brachyury(T) and HNF3β (Wilkinson et al., 1990; Sasaki et al., 1993). None of these mesoderm markers was expressed in any of the mutant embryos examined, whereas control embryos displayed the characteristic expression patterns of these genes (Figs. 4A–4D). Therefore, these results further confirmed that no mesoderm had been formed in the BMPR-II mutant embryos.

Although none of the mutant embryos expressed T gene, it is important to test whether the A-P axis is established in the BMPR-II mutant embryos, since the anterior-posterior (A-P) axis is established at the egg cylinder stage prior to formation of the primitive streak (reviewed in Beddington and Robertson, 1999). We therefore examined the expression pattern of Cripto (Ding et al., 1998). Cripto plays a critical role in A-P axis formation in mouse embryos. Cripto is initially expressed uniformly in the epiblast at E5.5, but before gastrulation its transcript becomes restricted to the region of the proximal rim of the epiblast that will give rise to the primitive streak (Ding et al., 1998). Cripto localized to the region of the nascent primitive streak in normal embryos at E6.5, whereas it was expressed weakly in the proximal region of the epiblast in the BMPR-II mutant embryos (Figs. 4E and 4F). At E7.5, in contrast to the normal expression pattern of Cripto, we observed increased Cripto expression, which was symmetric and uniform in the epiblast of the BMPR-II mutant embryo (Fig. 4G). These results suggest that differentiation of the epiblast of mutant embryos might have been impaired and the A-P axis was not properly established.

Expression of the Tissue-Specific Genes for Visceral Endoderm in BMPR-II Mutant Embryos

Visceral endoderm (VE) has been shown to play important roles prior to and during gastrulation. In Smad4 and ALK2 mutant embryos, gastrulation was disrupted due to impaired differentiation of VE (Sirard et al., 1998; Gu et al.,
FIG. 3. Gross morphology and histological analysis of the BMPR-II mutant embryos. Wild-type (A, C, E) and BMPR-II mutant (B, D, F) embryos at E6.5 (A, B), E7.5 (C, D), and E8.5 (E, F) are shown. The mutant embryos appear to be arrested prior to gastrulation. Bar, 200 µm. Sagittal sections of normal (G, I) and mutant (H, J) embryos at E6.5 (G, H) and E7.5 (I, J) are shown. am, amnion; ch, chorion; ee, embryonic ectoderm; exe, extraembryonic ectoderm; me, mesoderm; pe, parietal endoderm; ve, visceral endoderm.

FIG. 4. Expression of markers of primitive streak or mesoderm and Cripto in the BMPR-II mutant embryos at E7.5. (A and B) Brachyury(T) is expressed along the entire primitive streak in the wild-type embryo (A), but not in the mutant embryo (B). (C and D) HNF3β is expressed in the anterior region of the primitive streak and in the node in the wild-type embryo (C), but not in the mutant embryo (D). (E–G) Expression of Cripto in the primitive streak (ps) in the E6.5 wild-type embryo (E), in the proximal region (arrow) of epiblast in the E6.5 mutant embryo (F), and in the entire epiblast (ep) in the E7.5 mutant embryo (G). Bar, 200 µm.
control reaction without reverse transcriptase (RT) was performed using primers as probes. To assess the absence of DNA contamination, RT-PCR was performed on cDNAs from normal embryos (lanes 1–3), mutant embryos (lanes 4 and 5), and decidual tissue (lane 6). To normalize the amounts of total RNAs used as starting materials, the cDNA of HPRT was amplified. Verification of the PCR products was assessed by Southern blot analysis by use of end-labeled internal probes. Similar amounts of total RNA were reverse transcribed, as shown by relatively equivalent amounts of PCR products generated with HPRT primers (data not shown). Similar amounts of total RNA were reverse transcribed, as shown by relatively equivalent amounts of PCR products generated with HPRT primers (Fig. 5; HPRT).

**FIG. 5.** Semiquantitative RT-PCR analysis of tissue-specific genes for visceral endoderm in E7.5 mutant embryos. Total RNAs were extracted from normal embryos (lanes 1–3), mutant embryos (lanes 4 and 5), and decidual tissue (lane 6). To normalize the amounts of total RNAs used as starting materials, the cDNA of HPRT was amplified. Verification of the PCR products was assessed by Southern blot analysis by use of end-labeled internal probes. To assess the absence of DNA contamination, control reaction without reverse transcriptase (RT) was performed (lane 1).

To evaluate the differentiation of VE of BMPR-II mutant embryos, we examined the expression of some VE-specific genes by whole-mount in situ hybridization and RT-PCR (Duncan et al., 1997; Sirard et al., 1998). The expression of HNF4 in the BMPR-II mutant embryos was remarkably reduced or undetectable by whole-mount in situ hybridization (data not shown). Total RNAs were extracted from three normal and two mutant embryos at E7.5 and from decidual tissue, and the genotype of each embryo was confirmed by RT-PCR using BMPR-II-specific primers (data not shown). Similar amounts of total RNA were reverse transcribed, as shown by relatively equivalent amounts of PCR products generated with HPRT primers (Fig. 5; HPRT). We found that all VE-specific genes were expressed in BMPR-II mutant embryos at E7.5, although the expression levels of HNF1 and HNF4 in mutant embryos were slightly lower than those in normal embryos (Fig. 5). These results suggest that differentiation of VE is relatively normal in BMPR-II mutant embryos.

**DISCUSSION**

Members of the TGF-β superfamily, their receptors, and their intracellular signaling molecules, Smads, have been shown to play important roles in the development of vertebrates and invertebrates. BMPs are essential for early development and organogenesis in mice (reviewed in Hogan, 1996). In this study, we have shown that BMP signaling through BMPR-II is required during early mouse development.

**Expression of Type II Receptors for BMPs and Functional Redundancy in Early Mouse Embryos**

In contrast to ActR-II and ActR-IIB, which serve as type II receptors for BMPs and activins, BMPR-II specifically binds BMPs, but not activins (Rosenzweig et al., 1995; Liu et al., 1995; Ohno et al., 1995). Expression of the three different type II receptors for BMPs, and that of the three BMP type I receptors, may be strictly regulated spatially and temporally, and these receptors may have certain functional redundancy as well as nonoverlapping functions in BMP signaling during mouse embryogenesis. It is thus important to determine the expression of BMPR-II and compare it with that of ActR-II and ActR-IIB.

BMPR-II mRNA is expressed in various adult tissues. The expression patterns of BMPR-II have been examined in pre- and postimplantation mouse embryos by RT-PCR and in situ hybridization; its transcript was detected in one-cell, two-cell, and blastocyst stage embryos, E6.0 embryos, and all three germ layers of E7.5 embryos ubiquitously (Roelen et al., 1997, and the present study, Fig. 1).

Expression of ActR-II and ActR-IIB is also observed in embryos from E5.5 to E7.5 (Manova et al., 1995). Genetic studies have shown that embryos lacking either ActR-II or ActR-IIB develop to term with no gross defects in mesoderm formation (Matzuk et al., 1995; Oh et al., 1997). However, double mutant embryos for ActR-II and ActR-IIB show abnormal development during gastrulation (Song et al., 1999). These results suggest that functional redundancy exists between ActR-II and ActR-IIB in early mouse development. In contrast, our present results from the BMPR-II mutant embryos suggest that BMPR-II may have unique functions during early mouse embryogenesis, which cannot be physiologically compensated for by ActR-II or ActR-IIB.

**BMPR-II Functions in Egg Cylinder Elongation and Mesoderm Formation**

Our present results show that BMPR-II is necessary for egg cylinder elongation. The BMPR-II mutant embryos failed to undergo normal gastrulation and were arrested at the egg cylinder stage without inducing any mesoderm. Among the previously reported mutant embryos lacking genes involved in BMP signaling pathways, BMP4, ALK2, ALK3, Smad4, and Tlx-2 mutant embryos exhibit gastrulation defects with impaired mesoderm formation (Winnier et al., 1995; Gu et al., 1999; Mishina et al., 1995; Sirard et al., 1998; Yang et al., 1998; Tang et al., 1998). Among them, the phenotypes of BMPR-II mutant embryos appear to be similar to those of the BMP4, ALK3, and Smad4 mutant embryos. The E6.5 ALK3 mutant embryos were able to differentiate into some mesodermally derived cell types when grown ectopically in testes, suggesting that ALK3 is not absolutely required for mesoderm formation (Mishina et al., 1995). In addition, aggregation of mutant Smad4 ES cells with wild-type tetraploid morulae rescued the gastrulation defect of Smad4 mutant embryos (Sirard et al., 1998), indicating that Smad4 is initially required for the differen-
tiation of the visceral endoderm and that the gastrulation defect in the epiblast is secondary and non-cell autonomous. However, it is not clear whether the gastrulation defect of BMPR-II mutant embryos resulted from abnormal visceral endoderm differentiation, because all genes assayed for visceral endoderm differentiation were expressed in BMPR-II mutant embryos (Fig. 5). Further studies will be required to determine whether the gastrulation defect in the BMPR-II mutant embryos results from disruption of the BMPR-II signaling pathway in epiblast or extraembryonic tissues.

A-P Axis Formation in the BMPR-II Mutant Embryos

Recent studies have shown that precocious anterior patterning in the extraembryonic endoderm of the mouse embryos is established before there is any sign of primitive streak formation, and then the anterior visceral endoderm (AVE) induces anterior character in the underlying epiblast. Anterior pattern is therefore required for complete axis formation before the primitive streak is formed (reviewed in Beddington and Robertson, 1999). Cripto encodes a secreted protein with cysteine-rich and epithelial growth factor-like motifs. Cripto mutant embryos do not orient their A-P axis correctly, and AVE markers such as Hex remain located distally (Ding et al., 1998). Hence, the Cripto protein, secreted by the epiblast, is necessary for the cell movements in the visceral endoderm and epiblast that orient the A-P axis. In the BMPR-II mutant embryos at E6.5, Cripto presumably starts to be expressed in the proximal rim of epiblast (Fig. 4F). Interestingly, we observed increased Cripto expression, which was symmetric and uniform in the epiblast of the BMPR-II mutant embryo at E7.5 (Fig. 4G). The homeobox gene Hex is an AVE marker and is expressed initially in the primitive endoderm of the implanting blastocyst, but by E5.5 its transcripts are present only in a small patch of visceral endoderm at the distal tip of the egg cylinder. The Hex-expressing cells give rise only to anterior progeny, which populate the AVE (Thomas et al., 1998). We observed that the expression of Hex in BMPR-II mutant embryos was detectable by RT-PCR but not by whole-mount in situ hybridization (data not shown). These results suggest that the A-P axis is probably not established in the BMPR-II mutant embryos.

In the ALK2 mutant embryos, the A-P axis is established as shown by Hesx1 and T expression on the opposite sides of the E8.5 mutant embryos (Gu et al., 1999). The defects in the ALK2 mutant embryos are thus less severe than those in the mouse embryos lacking either ALK3 or BMPR-II. Taken together, we suggest that the signals of BMPs, especially those of BMP4, during egg cylinder growth and A-P axis formation might be transduced mainly through a heteromeric complex of BMPR-II and ALK3/BMPR-IA.

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