Available online at www.sciencedirect.com
SCIENCE DIRECT
Cytokine & Growth Factor Reviews 16 (2005) 265–278
Cytokine & Growth Factor Reviews
www.elsevier.com/locate/cytogfr

BMP signaling and early embryonic patterning

Satoshi Kishigami [1], Yuji Mishina *

Molecular Developmental Biology Section, Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA

Available online 24 May 2005

Abstract

Bone morphogenetic proteins (BMPs) play pleiotropic roles during embryonic development as well as throughout life. Recent genetic approaches especially using the mouse gene knockout system revealed that BMP signaling is greatly involved in early embryonic patterning, which is a dynamic event to establish three-dimensional polarities. The purpose of this review is to describe the diverse function of BMPs through different receptor signaling systems during embryonic patterning including gastrulation and establishment of the left–right asymmetry.

Keywords: TGF-β; BMP; Receptor; Embryogenesis; Gastrulation; Embryonic patterning

1. Introduction

Bone morphogenetic proteins (BMPs) were originally identified by their ability to cause bone differentiation about 40 years ago [1]. Because this bone inducing activity was sensitive for protease digestion, the activity was named as bone morphogenetic protein. Together with *decapentaplegic*, *60A*, *screw* (*Drosophila*), *Vg1* (frog), *Dorsalin-1* (chick), *Univin* (Ser urchin), *Daf-7* (*Caenorhabditis elegans*), growth and differentiation factors (GDFs), *nodal* and *lefty*, BMPs compose a large subgroup within the transforming growth factor-β (TGF-β) gene superfamily [2,3] (Fig. 1). Most of these BMPs are capable of inducing the formation of bone when subcutaneously implanted into rodents [2,4]. Bone formation occurs through a series of endochondral events initiated by chemotaxis of mesenchymal stem cells into the implantation site [5,6]. Thus, the BMP family of proteins possesses potent bone inducing properties. However, recent studies of several organisms demonstrate that several BMPs have other roles during embryogenesis, notably in dorsoventral and/or anterior–posterior axis formation [7–9]. In *Drosophila melanogaster*, mutations in *decapentaplegic* (*dpp*), which is a homologue of BMP2 and BMP4, cause dorsoventral patterning abnormalities at the blastoderm stage [10]. In *Xenopus leavis*, BMP4 can act as a posterior-ventralizing factor in animal cap explant and blastocoele implant assays [11,12]. These findings prompted mouse geneticists to study the function of BMPs during mouse development in addition to their function on skeletogenesis. In the last decade, functions of many of BMP ligands, their receptors and signaling molecules were investigated in the mouse using transgenic and knockout techniques [3,9]. In this article, we will focus on the function of BMPs and related molecules that are critical during early mouse development to determine morphological events of mouse embryos.

2. BMPs and their signaling

As BMPs are secreted proteins, characterization of their receptors and signal transduction pathways is an important step in understanding the role of these proteins in development. Upon ligand binding, both types I and II receptors form hetero-multimers [13]. Type II receptors phosphorylate a short stretch of amino acids called a GS box in type I receptors to activate their kinase activity, then type I

* Corresponding author. Tel.: +1 919 541 1095; fax: +1 919 541 3800.
E-mail addresses: kishigami@cdb.riken.go.jp (S. Kishigami), mishina@niehs.nih.gov (Y. Mishina).
[1] Present address: RIKEN Center for Developmental Biology, 2-2-3 Minatojima-Minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan. Tel.: +81 78 306 0111; fax: +81 78 306 0101.

1359-6101/$ – see front matter
doi:10.1016/j.cytogfr.2005.04.002

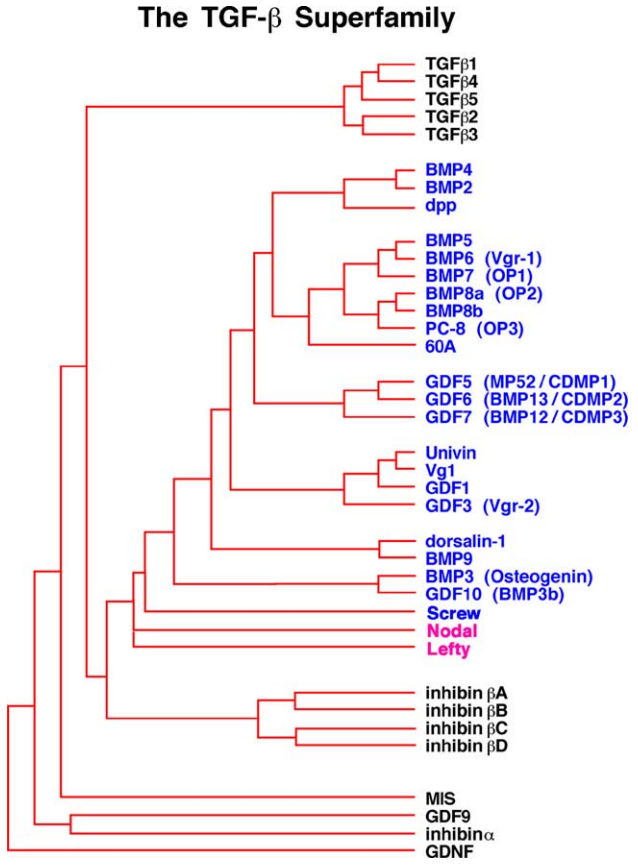

Fig. 1. TGF-β superfamily and BMP subfamily. BMPs compose a large subfamily within the TGF-β superfamily (blue). Signaling mechanisms of some of the BMPs, particularly these belong to the dpp family (BMP2, BMP4) and the 60A family (BMP5–8), have been extensively investigated. Nodal and Lefty (pink), which are distal members of BMP subfamily, utilize distinct signaling pathways that are similar to these of TGF-β and activins (see Fig. 2).

receptors phosphorylate downstream targets such as smads [8,14] (Fig. 2). In vertebrates, seven type I receptors and five type II receptors have been found so far. Type II receptors are named by their ligands, i.e., ActRIIA (activin type II A receptor), BMPRII (BMP type II receptor), whereas type I receptors frequently referred as ALK (Activin receptor like kinase), i.e., ALK1, ALK2 [15]. Three type I receptors (BMP type I A receptor, BMP type I B receptor and ACVRI) and three type II receptors (BMPRII, ActRIIA and ActRIIB) behave as receptors for BMPs (Table 1). ACVRI was originally found as an activin receptor, but it is now believed to be a receptor for BMPs. The specificity of signaling is primarily determined by type I receptors [16]. The specificity of ligand binding is, however, altered by the combination of types I and II receptors [17]. These combinations and ligand specificity are summarized in Table 1.

Activated type I receptor kinases phosphorylate Smads (R-Smads) and subsequently, phospho-Smads form heterodimers with Smad4 for nuclear translocation to alternate gene expressions (Fig. 2) [14,18]. Type I receptors for BMPs phosphorylate Smad1, Smad5 and Smad8, whereas type I receptors for TGF-β, Activin and Nodal phosphorylate Smad2, and Smad3 (Table 1). Several proteins including SARA and Hgs play important roles during formation of receptor–Smad complex [14,18]. In addition to the Smad pathway, BMPs are believed to activate several different kinase pathways including MAPK, PI3 kinase and PKC [18].

Expression of BMP receptors during early embryogenesis is rather broad compared with that of BMP ligands (see below). Besides expression pattern and levels of BMP ligands and receptors, there are several other classes of gene products that could regulate BMP functions as modifiers of BMP signaling. Cystein-rich extracellullar proteins such as Noggin and Chordin bind to BMP ligands to prevent their interaction to the receptors [19,20]. Overexpression studies in Xenopus embryos revealed that inhibition of BMP signaling by these proteins caused over proliferation of neural tissues [21]. Loss of function studies in mouse, however, showed relatively less dramatic changes especially during early embryogenesis suggesting that there would be redundant functions among BMP binding proteins [22–26]. Intensive studies including generation of double/triple knockout mice of these genes are taking place in order to address this issue.

3. BMP and gastrulation

3.1. How mouse embryos gastrulate

Shortly after implantation, the mouse embryo shows one polarity, the initial proximal–distal (P–D) polarity, which has been established around embryonic day 3.5 (E3.5) [27–30]. This stage of embryos can be divided in two regions, the embryonic region consisted with epiblast (embryonic ectoderm) and the extraembryonic region consisted with visceral endoderm, parietal endoderm and extraembryonic ectoderm [31] (Fig. 3). The embryonic region will give rise to the fetus while the extraembryonic region together with maternal tissues will give rise to placenta. Recent studies indicate that the extraembryonic tissues (the visceral endoderm and the extraembryonic ectoderm) of the mouse embryo play critical roles during gastrulation. Prior to the gastrulation (E5.5–E6.0), one group of cells in the distal tip of the visceral endoderm (distal visceral endoderm, DVE) starts to move one direction to form a prospective signaling center called as the anterior visceral endoderm (AVE) [32–34] (Fig. 3). Gastrulation begins at E6.5, when the primitive streak, a mesodermal structure, forms in the posterior epiblast at its junction with extraembryonic ectoderm. Therefore, the initial P–D polarity converted to the anterior–posterior (A–P) polarity, and this process is called as axis rotation [31] (Fig. 3).

As gastrulation progresses, cells are recruited to intercalate between the proximal and the distal ends of

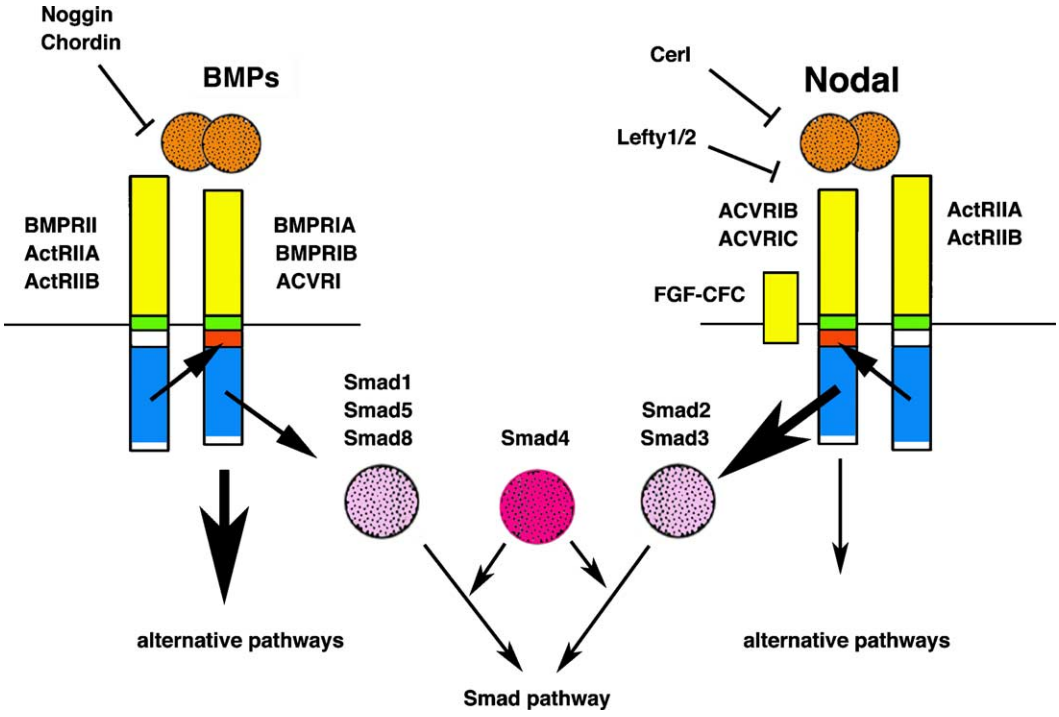

Fig. 2. How BMP signaling is regulated. TGF-β superfamily members bind to types I and II receptors that are membrane bound Ser/Thr kinases. Upon ligand binding, the kinase domain of a type II receptor (blue) phosphorylates the GS box (red) in a type I receptor to activate. BMPs and Nodal use different type I receptors to send signals to different Smad proteins. Cys-rich extracellullar proteins such as Noggin and Chordin directly bind to BMPs to prevent their receptor binding. Cerl binds to Nodal, and Lefty 1/2 binds to Nodal and FGF-CFC, which is a co-receptor for Nodal to antagonize Nodal signaling. During early embryogenesis, the Smad pathway seems to be a major pathway for Nodal whereas alternative pathways such as MAPK pathway seem to be a major pathway for BMPs.

the primitive streak, and ultimately the primitive streak extends to the distal tip of the egg cylinder [27,29]. Very little is known about the mechanisms that regulate recruitment of epiblast cells to the primitive streak during gastrulation. Several growth factors including BMPs are expressed during gastrulation, and based on their expression studies and genetic studies, some of them are believed to be essential for initiation of gastrulation and patterning of mesoderm [9] (Fig. 3).

3.2. Roles of BMPs in the extraembryonic region before and during gastrulation

Asymmetric cell movement in the extraembryonic region prior to the gastrulation suggests that early patterning in the extraembryonic tissues is necessary to allow epiblast gastrulation via regional interactions. Indeed, it is shown that the cells in the anterior visceral endoderm signal to the epiblast to pattern the anterior neuroectoderm [32]. Recent studies indicate that BMP ligands and their signaling molecules expressed in the extraembryonic tissues of the mouse embryo contribute to the embryonic patterning. For these studies, two distinct approaches are frequently taken: one is to generate chimeric embryos using ES cells [35] and the other is to disrupt gene of interest in an epiblast-specific manner using a Cre-loxP system [36]. For the former case, ES cells exclusively contribute to the embryonic region; therefore, the origin of the extraembryonic region is the host embryo whereas the origins of the embryonic region are a combination of the host embryo and ES cells. Chimeric embryos generated between a wild type embryo and ES cells that are homozygous mutant for one gene would show less

Table 1
Nomenclature of TGF-β superfamily receptors and their signaling properties

Symbol	Synonyms	Chromosome	Type II partners	Ligands	Smads
Bmpr1a	*Alk3, Bmpr, TFR11, Brk1*	14	*Bmpr2, Actr2a, Amhr2*	BMP2, 4, GDF6, MIS	1/5/8
Bmpr1b	*Alk6, CFK-43a, Brk2*	3	*Bmpr2, Actr2a, Actr2b*	BMP2, 4, 7, GDF5, 6	1/5/8
Acvr1	*Alk2, ActRI, ActRIA, Alk8, SKR1, Tsk7L*	3	*Bmpr2, Actr2a*	BMP2, 4, 7	1/5
Acvr1b	*Alk4, ActRIB, SKR2*	15	*Actr2a, Actr2b*	Activin, Nodal	2/3
Acvr1c	*Alk7*	2	*Unknown*	Nodal	2/3
Acvrl1	*Alk1*	15	*Tgfbr2*	TGF-β	1/5/8
Tgfbr1	*Alk5, TbetaRI*	5	*Tgfbr2*	TGF-β	2/3

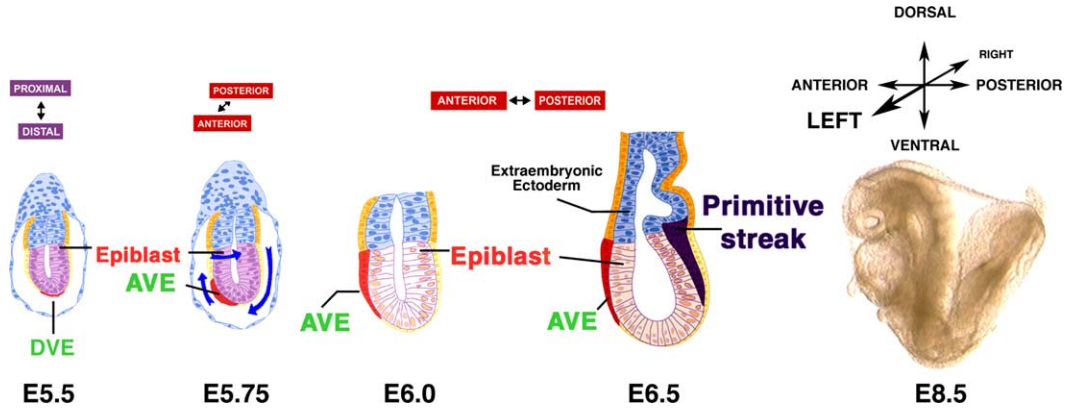

Fig. 3. Schematic representation of early mouse development, Shortly after implantation, mouse embryos establish the proximal–distal polarity. Epiblast (purple) is surrounded the visceral endoderm (pale yellow). One group of cells in the distal tip of the visceral endoderm (DVE) starts to move toward the future anterior part to form the anterior visceral endoderm (AVE). Around E6.5, gastrulation starts on the opposite side of AVE to form the primitive streak (dark blue). Thus, the initial proximal–distal polarity is converted to the anterior–posterior polarity. This process is called axis rotation. Proximal–distal polarity at E6.5 now corresponds to the dorsal–ventral polarity. At this stage, there is no overt asymmetry in the left–right axis, and embryos establish the left–right asymmetry by E8.5. Adopted from [31].

severe phenotype than knockout embryos, if functions of the gene were critical in the extraembryonic region because the extraembryonic region is wild type in this combination.

3.2.1. Functions of BMP ligands in the extraembryonic tissues

Bmp4 is highly expressed in the extraembryonic ectoderm as well as primitive streak before and during gastrulation [37–40]. The posterior region of the primitive streak in the *Bmp4* mutant embryos shows a ventrally projected bulge, but this abnormality is rescued in tetraploid chimeras using *Bmp4* mutant ES cells and wild type embryos [41]. In the tetraploid chimeric embryos, all of the cells in the embryonic region are derived from ES cells [42], while cells in the extraembryonic region are wild type. This result suggests that the expression of *Bmp4* in the

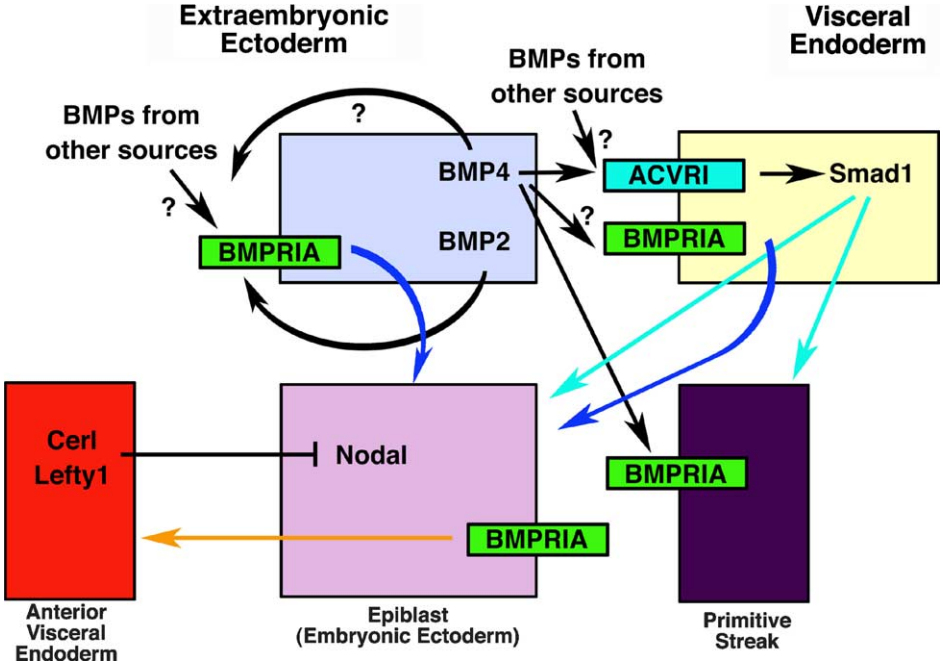

Fig. 4. Model for BMP function in the extraembryonic and embryonic region prior to and during gastrulation. BMP4 that is expressed in the extraembryonic region is critical for normal primitive streak formation, presumably signaling through BMPRIA. In the visceral endoderm, BMP4 (and potentially other BMPs) signals through ACVRI, then subsequently to Smad1, to trigger unknown signals that is important for completion of gastrulation and PGC formation (light blue arrows). BMP2 is critical for normal development of the extraembryonic tissues. BMPRIA signaling in the extraembryonic ectoderm and the visceral endoderm induces unknown signaling that is critical for epiblast to initiate gastrulation (blue arrows). BMPRIA signaling in the epiblast is important for proper development of the anterior visceral endoderm (an orange arrow). The anterior visceral endoderm expresses Cerl and Lefty1 that antagonize Nodal activity in the anterior side of the epiblast to restrict formation of the primitive streak in the posterior side.

extraembryonic ectoderm, not in the primitive streak, is required for normal formation of the primitive streak (Fig. 4).

Bmp2 starts to be expressed at E6.0 in the extraembryonic region [40], then in the extraembryonic mesodermal cells lining the chorion and amnion after gastrulation [43]. *Bmp2* mutant embryos can gastrulate, but they show abnormal development in the extraembryonic region such as failure of the fusion of the preammniotic canal and die by E8.5 [43]. Interestingly, the heart is developed in a presumptive exocoelomic cavity suggesting that BMP2 is important for proper development of the extraembryonic tissues to influence the position of the heart [43]. However, it is still unclear the importance of the expression domain of BMP2 in the extraembryonic region. Generation of *Bmp2* mutant ES cells and chimeric analyses using these cells are awaited.

3.2.2. Functions of BMP signaling through BMPRIA in the extraembryonic tissues

Although it is possible that BMP ligands expressed in the extraembryonic region interact with receptors in the embryonic region, disruption of BMP receptors in the extraembryonic region can directly address the functional importance of BMP signaling in the extraembryonic region. *Bmpr1a* that encodes a type I receptor for BMP2 and 4 is expressed ubiquitously in the epiblast and the extraembryonic region at the gastrulation stage embryos [44]. Disruption of *Bmpr1a* causes early embryonic lethality without mesoderm formation [44]. Unlike Nodal mutant embryos (see below), no expression of *Brachyury* and no migration of DVE were observed [44] (and unpublished observation).

To rescue the early lethality, a floxed allele for *Bmpr1a* was generated [9,45]. The *Mox2-Cre* (*MORE*) mouse line is a Cre transgenic line that expresses Cre recombinase in an epiblast-specific manner [46]. Breeding of the floxed allele of *Bmpr1a* with *MORE* should generate mosaic embryos of wild type extraembryonic region and mutant embryonic region of *Bmpr1a*. Interestingly, the mutant embryos do not show overt morphological abnormality up to E7.5 and initiates gastrulate normally [47]. This suggests that BMP signaling through BMPRIA plays a critical role in the extraembryonic region before (and during) gastrulation (Fig. 4).

Since homozygous mutant ES cells for *Bmpr1a* could not be established from homozygous mutant blastocysts [48], function of BMPRIA signaling in the extraembryonic region was not investigated until recently. Interestingly, BMP4 is now shown to be an essential factor to maintain pluripotency of mouse ES cells [48,49]. More excitingly for the people in the BMP field, BMP4 signals through BMPRIA to suppress p38 MAP kinase activity and this is the molecular mechanism for maintenance of pluripotency in the ES cells [48]. Based on this insight, homozygous mutant blastocysts for *Bmpr1a* were cultured with a MAP kinase inhibitor, and homozygous mutant ES cells for *Bmpr1a* were established [48]. Availability of the mutant ES cells should prompt the detailed analyses of BMPRIA function during gastrulation and organogenesis.

3.2.3. Functions of BMP signaling through ACVRI in the extraembryonic tissues

Unlike *Bmpr1a* that is expressed ubiquitously in the gastrulation stage embryos *Acvr1* is expressed weakly only in the visceral endoderm at the stage of gastrulation [50–52], subsequently expressed in embryonic portions such as node, midline, head mesenchyme and heart primodia [50,52,53]. Targeted disruption of *Acvr1* causes embryonic lethality at the stage of gastrulation with less severe phenotype than that of *Bmpr1a* [50,54]. Gastrulation is initiated and mesodermal wings are formed, but their development is arrested at mid/late streak stage. The gastrulation defect is rescued in chimeric embryos generated by injection of homozygous mutant ES cells for *Acvr1* to wild type blastocysts [50,54]. This indicates that BMP signaling through ACVRI plays critical roles in the extraembryonic region prior to, and during gastrulation (Fig. 4).

Recently, it is shown that BMP signaling through ACVRI in the visceral endoderm is necessary for normal induction of primordial germ cells (PGCs) in the epiblast [55]. There is a known absence of PGCs in *Bmp4* mutant embryos [38,56] and reduction of PGCs in *Bmp8b* or *BMP2* mutant embryos [39,40]. These findings allow hypothesizing that these BMP ligands expressed in the extraembryonic ectoderm signal into the visceral endoderm through ACVRI, then the visceral endoderm sends unidentified signal to the epiblast for initiation of PGC formation. A conditional allele of *Acvr1* that floxed exon 7 has been established recently [57]. Visceral endoderm-specific disruption of *Acvr1* should provide some of the answers for molecular identities of downstream targets in the visceral endoderm (Fig. 4).

3.2.4. Other BMP related molecules

Bmpr2 encodes the type II BMP receptor that forms heteromers with BMPRIA, BMPRIB, or ACVRI [17]. Disruption of *Bmpr2* causes severe embryonic lethality that mimics the abnormalities of *Bmpr1a* mutant embryos [58]. Mutant embryos fail to gastrulate and no signs of mesoderm formation are observed [58]. Interestingly, the mutant embryos still express visceral endoderm markers such as *HesX1*, but no spatial expression pattern was looked for [58]. These observations suggest that BMPRII and BMRIA form heteromultimer to transduce BMP signaling at the stage of gastrulation, presumably in the extraembryonic region. Detailed analyses including special pattern of gene expression in the mutant embryos is awaited.

S*mad1* is one of the major downstream targets of BMP receptor complexes. *Smad1* is highly expressed in the visceral endoderm at E6.5, and subsequently expressed in both embryonic and extraembryonic tissues after gastrulation [59]. Mutant embryos for *Smad1* show ruffles in the visceral endoderm adjacent to the primitive streak [59,60].

Embryos die by E10.5 due to the failure of chorioallantoic fusion and these abnormalities cannot be rescued by a wild type epiblast. These abnormalities well support the above hypothesis that BMP signaling in the extraembryonic region is indispensable for normal gastrulation.

3.2.5. Function of Nodal during gastrulation

Nodal is a distant member of the BMP subfamily and utilizes different signaling pathways, ACVRIB (ALK4) or ACVRIC (ALK7) in combination of ActRIIA or ActRIIB, and downstream targets, Smad2 and Smad3 [17,61]. Nodal was originally disrupted by retroviral insertion in ES cells, and the disrupted gene was then identified as a member of TGF-β superfamily [62]. Mutant embryos die without overt gastrulation; however, unlike *Bmpr1a* mutant embryos, the embryos form randomly positioned patches that express *Brachyury* [63]. Results from detailed analyses of several different types of targeted mutations of *Nodal* suggest the model that Nodal is required for establishing the initial P–D polarity, which then converted into A–P polarity during movement of AVE [63–65]. However, expression of *Nodal* in the visceral endoderm is not necessary for axis rotation. Reciprocal signaling interaction between the extraembryonic ectoderm and the epiblast plays a critical role for axial rotation [65,66]. Since AVE expresses Lefty1 and Cerberus-like (Cerl) and both antagonize Nodal signaling [67,68], it is suggested that Lefty1 and Cerl from AVE restrict Nodal activity to the posterior end of the embryos for primitive streak formation. Recently, it is suggested that direction of the migration of DVE (that will be future anterior) is determined by unequally stimulated proliferation of the cells in the VE by Nodal signaling [69]. Functions of Nodal are divergent both in the extraembryonic and embryonic regions during gastrulation, but several difference in the phenotypes of mutant embryos suggest that BMP signaling through BMPRIA may play distinct/parallel roles from that of Nodal, at least in part.

3.3. Embryonic patterning in germ layer development during and after gastrulation

After initiation of the formation of primitive streak, primitive streak elongates to reach the distal tip of the epiblast. Cell fate mapping studies revealed that timing and position of cells entering the to the streak is critical for mesoderm specification; for example, cells enter the most proximal part of the streak (and at the earliest timing) give rise the extraembryonic mesoderm (Fig. 5). Recent genetic studies revealed that divergent and critical role of BMPs and other TGF-β superfamily members during these processes.

3.3.1. Functions of BMP ligands for germ layer development

Homozygous mutant embryos for *Bmp4* show multiple defects after gastrulation such as eye and germ cells to die by E10.5 [37–39,70]. Depending on the genetic background,

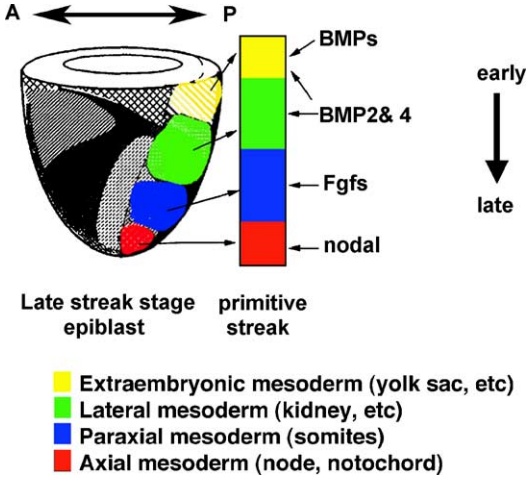

Fig. 5. Model for BMP and Nodal functions on A–P patterning during gastrulation. At early to mid-streak stage (E6.5–7.0), mainly extraembryonic mesodermal cells are recruited to the primitive streak (yellow). Precursors of heart mesoderm and cranial mesenchyme are also recruited during mid-streak stage. At late streak stage, prospective lateral and paraxial mesoderm cells are recruited to the primitive streak (green and blue). The node is specified at the distal end of the primitive streak that gives raise axial mesoderm. BMPs play major roles for differentiation of the extraembryonic, lateral and heart mesoderm whereas Nodal plays critical roles for differentiation of the axial mesoderm. Genetic studies suggest that FGF signaling is important for the development of paraxial mesoderm.

mutant embryos develop up to 20 smite stage, but they show considerable defects in posterior part of the body and fail to develop allantois [37,38]. Chimeric analysis using tetraploid embryos revealed that *Bmp4* expression in the extraembryonic mesoderm is not essential for initiation of allantois development, but is essential for the differentiation of allantois and survival of PGCs [71]. The chimeric analyses also reveal that *Bmp4* expression in the extraembryonic ectoderm is essential for formation of PGCs in the epiblast [71].

As mentioned above, *Bmp2* is expressed in the extraembryonic mesoderm and the homozygous mutant embryos show abnormal structure in the extraembryonic region [43]. Like *Bmp4* mutant embryos, they show various phenotypes partly due to the genetic background, but none of them can survive beyond E9.5. They are small and never turned, however, can develop somites [43]. Thus, even though BMP2 and BMP4 share more than 90% of their identity in their amino acid sequence, the phenotype of BMP2 homozygous mutant embryos are somewhat different from that of BMP4 mutant embryos, probably due to the difference of their expression pattern or utilization of their receptor complex. Chimeric analyses should address the importance of BMP2 function during mesodermal patterning as well as organogenesis.

3.3.2. Functions of BMP receptors for germ layer development

Homozygous mutant embryos for *Bmpr1a* die without mesoderm formation, but as mentioned above, conditional

mutant embryos using an epiblast-specific Cre transgenic mouse line can gastrulate to develop mesodermal tissues [47]. These embryos show interesting A–P patterning defects such as expansion of anterior neural ectoderm at the expense of surface ectoderm. Overall A–P and D–V pattern in neural ectoderm is normal in some extent [47]. Interestingly, the prechordal plate, the rostral-most mesendoderm that is derived from the axial mesoderm, is expanded in the mutant embryos underlying the enlarged portion of the anterior neural tissue. AVE is also expanded, even though cells in the visceral endoderm of the mutant embryos are still wild type for *Bmpr1a* [47]. These findings suggest that BMP signaling through BMPRIA in the epiblast negatively regulates expansion of AVE and that in the anterior mesendoderm negatively regulates expansion of the prechordal plate. This idea well fits with the double assurance model for anterior neural patterning in the mouse that claims forebrain fate is promoted by the AVE and reinforced and maintained by the anterior axial mesoderm [32,72].

As mentioned above, the gastrulation defect of *Acvr1* is rescued in chimeric embryos with wild type extraembryonic tissues and they can survive several more days in utero [50,54]. High contribution chimeras (more than 80% of the cells in the epiblast and its derivatives are homozygous mutant for *Acvr1* while all cells in the extraembryonic tissues are wild type) show turning defects and smaller posterior tissues that resembles to the Bmp4 mutant embryos [50,53,54]. Chimeras less than 80 % of the contribution of *Acvr1* null ES cells develop relatively normal [53]; however, preliminary studies revealed that the mutant cells were excluded from several tissues suggesting cell autonomous function of ACVRI signaling during organogenesis (unpublished results).

Expression of *Bmpr1b* is firstly observed at E9.5 embryos [73] and mutant embryos for *Bmpr1b* show defects in differentiation and proliferation of chondrocytes around E13.5 [74,75]. These suggest that there would be minimal contribution of BMP signaling through BMPRIB during early stage of mouse development, however, it is still possible that redundant functions of BMPRIA and/or ACVRI signaling rescue the loss of BMPRIB signaling during establishment of body pattern. Generation of double or triple knockout of these receptor genes should uncover possible redundant functions during early embryogenesis.

3.3.3. Functions of Nodal for embryo patterning

In chimeric embryos generated by injection of wild type ES cells to *Nodal* mutant blastocysts, the gastrulation defect was rescued if contribution of the ES cells is over 30%, but embryos show anterior truncations [66]. Mutant embryos that still express limited amount of Nodal can undergo gastrulation with anterior–posterior patterning defects [76]. An epiblast-specific disruption of *Nodal* results morphologically identical embryos to *Nodal* null embryos. Based on the comparison of the phenotype among these embryos, it is suggested that low levels of Nodal expression in the epiblast is required for DVE formation, high levels of Nodal are required for axis rotation and specification of the anterior mesendoderm [77] (Fig. 5).

The above hypothesis is reinforced by the conditional disruption of Smad2, one of the major downstream targets of Nodal signaling. Mutant embryos for *Smad2* cause patterning abnormalities during gastrulation, but unlike *Bmpr1a* or *Bmpr2* mutants, they can form extraembryonic mesoderm [78,79]. Chimeric analyses reveal that Nodal signaling via Smad2 is critical in the VE during gastrulation [80]. An epiblast-specific disruption of *Smad2* does not cause overt abnormalities during gastrulation, but show anterior patterning defects [81]. These again suggest that graded Nodal signaling is critical for A–P patterning in the epiblast.

Smad3 is another signal transducer for Nodal; however, homozygous mutant mice for *Smad3* develop to term without overt morphological abnormalities [82–84]. Because expression domains of Smad2 and Smad3 mostly overlap during early embryogenesis [80], functional redundancy of these two transducers was investigated. Some of the compound heterozygous mutants for *Smad2* and *Smad3* show craniofacial abnormalities presumably due to the defects in the prechordal plate [77]. Embryos homozygous mutant for *Smad3* and heterozygous for *Smad2* show anterior truncation [85]. Double homozygous mutant embryos for both *Smad2* and *Smad3* lack mesoderm and fail to gastrulate, however, the epiblast-specific *Smad2* embryos that are also homozygous mutant for *Smad3* can gastrulate with defects in induction of axial and paraxial mesoderm [85]. These suggest that dosage of Nodal signaling is important for A–P patterning during early embryogenesis presumably affecting to cell fate decision.

3.3.4. Final dilemma—does BMP and Nodal signaling merge together?

Biochemical studies have been shown that Smad4 is a common factor for both BMP and Nodal/activin/TGF-β pathways [18]. Consistent with this idea, homozygous mutant embryos for Smad4 embryos show no mesoderm formation that is more severe phenotype than any other individual Smad mutation [86,87]. Chimeric studies reveal the importance of Smad4 function in the extraembryonic tissues [86]. However, an epiblast-specific disruption of *Smad4* brought unexpected outcomes. These embryos establish the primary A–P axis and gastrulation is initiated normally [88]. The embryos fail to show the anterior primitive streak such as node and notochord that lead to anterior truncation [88]. These phenotypes are very similar to these of *Nodal* and *Smad2*/*Smad3* mutant embryos suggesting that Smad4 is an essential component of the Nodal signaling pathway during gastrulation. Some, but limited BMP-dependent developmental processes such as PCG formation and formation of a functional heart are affected [88]. These suggested that Smad4 may be a major

transducer of BMP signaling in the extraembryonic tissues, but during and after gastrulation, other pathways would play major roles for BMP signaling [18] (Fig. 2).

In summary, BMP signaling is required in the extra-embryonic region before and during gastrulation, but the identity and source of the ligands need to be investigated further. Nodal signaling in the extraembryonic region by Nodal expressed in the epiblast is also important for the initiation of gastrulation. After gastrulation, BMP signaling plays divergent roles especially for early-induced mesoderm patterning (extraembryonic mesoderm, heart mesoderm, lateral mesoderm) (Fig. 5). This is in contrast to the Nodal function that is important for later induced mesoderm (axial mesoderm and paraxial mesoderm). The divergent function of BMP signaling can be explained, at least in part, by the divergent downstream signaling pathways in addition to the Smad pathway. Further analyses of these pathways in early mouse embryos should provide further insight for molecular understanding of the function of BMPs.

4. BMP function on establishment of left–right asymmetry

4.1. How the mouse determines the left–right asymmetry

During normal vertebrate development, the asymmetry of the left–right (L–R) axis is crucial in positioning and morphogenesis of internal organs. The process of L–R determination can be divided into four steps: the initial breaking of the L–R symmetry in/near the node; transfer of asymmetric signals from the node to the lateral plate mesoderm (LPM); establishment of L–R asymmetric expression of signaling molecule in the left LPM; and transformation of these L–R asymmetric signals into morphologic asymmetries in the visceral organs [89]. Even if there are important species differences in the molecular regulation of L–R patterning [90,91], primary players in L–R patterning seem to be conserved through vertebrates [91–93]. Especially, *Nodal* and *Pitx2* are conservatively expressed in the left-specific manner through evolution from Ascidians to mammals [93–96]. In addition to several members of TGF-β superfamily such as Nodal and Lefty [91,92], the accumulating evidence strongly suggest that BMP signaling also play crucial roles in L–R patterning in mammals [9].

In the mouse, breaking symmetry in the L–R axis occurs in or near the node, which is located in the rostral end of the primitive streak, at the late neural-fold stage [97]. Each of the cells on the ventral side of the node has a monocilia rotating rapidly in a clockwise direction, which generates a leftward flow, referred to as ‘nodal flow’, of the extraembryonic fluid in the node [98,99]. This nodal flow is suggested to directly determine L–R axis potentially by transporting a L–R determinant, even though its molecular mechanism is still unknown [100,101].

4.2. Fine-tuning of Nodal signaling and left sidedness

4.2.1. Nodal is the primary factor for left sidedness

Nodal is expressed symmetrically in both sides of the node around E7.5, but, at the 4–5 somite stage (around E7.75–8.0), the expression of *Nodal* becomes transiently asymmetric, with more *Nodal* expressing cells on the left than the right side [102,103]. Later, *Nodal* is expressed in the lateral plate mesoderm, but in a left side-specific manner. Recent genetic evidence indicates Nodal activity in both the node and LPM is required for normal L–R patterning as a determinant for left sidedness [81,104–106]. Further, Nodal activity produced in the node is known to be required for lateral plate *Nodal* expression [105,106]. Compound mutants of Nodal receptors and its downstream target, Smad2, show right isomerism, demonstrating that Nodal is a left determinant [78]. Homozygous mutant embryos for *Actr2b* show heterotaxia [107], and compound mutants of *Actr2a* and *Actr2b* show various degrees of L–R defects [108,109]. These combined results suggest that Nodal binds to ActRIIA or ActRIIB, and ACVRIB (ALK4) to signal Smad2 during establishment of L–R asymmetry. However, the mechanism whereby Nodal activity is asymmetrically propagated from the node to activate gene expression in cells of LPM remains unclear [110].

4.2.2. Fine-tuning of Nodal activity by other TGF-β members

GDF1, another TGF-β superfamily member, is required for the expression of *Nodal*, *Lefty2* and *Pitx2* in the LPM [111,112]. *Gdf1* is expressed in the node and the LPM. Nodal and GDF1 may cooperate to enhance activation of down stream targets since transduction of both signaling require the presence of a co-receptor of the EGF-CFC family like Criptic [113]. These three genes, *Nodal*, *GDF*, *Cryptic*, share the expression in the node and LPM, and phenotypes of their mutants [105,111,114,115].

Distal members of the BMP subfamily, Lefty1 and Lefty2, are also implicated in L–R patterning by antagonizing Nodal signaling [116,67,117]. *Lefty1* is induced in the notochord and presumptive floor plate (midline) by Nodal signaling in the LPM [118] and believed to form a ‘‘midline barrier’’ to prevent overflow of left-side signals to the right side [116]. The midline barrier is still conceptual, but it may explain the phenotype of *Lefty1* mutant embryos well, since *Lefty1* null embryos show bilateral expression of *Nodal* in the LPM [116]. Recently, it is shown that Lefty directly interacts with the EGF-CFC co-receptor and with Nodal itself, which results in inhibition of Nodal signaling by preventing the association of Nodal with the receptor complex [119–121]. Indeed, wider expression of *Nodal* is observed in the *Lefty2* mutant embryos [67,122]. Thus, both Lefty1 and Lefty2 are required for fine-tuning of Nodal activity to establish proper L–R asymmetry. Involvement of Lefties in BMP signaling has also been proposed [123–125].

However, the significance of the crosstalk between BMP and Lefty for L–R patterning remains unclear.

4.3. Multiple roles of BMP signaling in left–right patterning

4.3.1. Importance of BMPs for right-sidedness in chick and frog

Recent studies suggest that BMP signaling is a 'right determinant' in the chick and *Xenopus* systems [92]. In *Xenopus*, truncated ACVRI (ALK2) receptor expression in the right side of blastula elicits heart reversals and altered *Nodal* expression and, on the other hand, constitutively active ACVRI receptor expression in the left side of blastula elicits heart reversal [126]. These data define BMP signaling as important components of a right-sided laterality pathway that antagonizes the left sided Vg1 pathway in *Xenopus*. Interestingly, XLefty and XBMP4 are also shown to interact both synergistically and antagonistically in a context-dependent manner to enhance the differences between the right-sided BMP/ACVRI/Smad pathway and the left-sided Vg1/Nodal pathway [124]. In the chick, *Bmp4* is expressed in Hensen's node with the highest levels on the right to positively regulate *Fgf8* expression on the right side of the node. It also bilaterally expressed in the LPM to prevent initiation of *Nodal* expression in the right LPM [127,128]. On the other hand, BMP2 is shown to be a positive regulator of Nodal signaling in the LPM [129,130]. Thus, BMP signaling may exert different functions on tissue- and/or temporal-specific manner probably using different receptors and transducers during L–R formation. This idea needs to be investigated further using genetic methods.

4.3.2. Smad5 for right-sidedness

In mammals, until recently little was known about BMP function in the development of L–R asymmetry, particularly the identity of the receptor(s) that are involved in L–R asymmetry [89]. This is primarily due to the fact that null mutants show embryonic lethality too early to examine their functions in the L–R patterning. The first evidence of involvement of BMP signaling in mammals came out in the study of *Smad5* mutant embryos. The *Smad5* mutants have defects in heart-looping and embryonic turning, which are the first signs of L–R asymmetry in mice. In the absence of Smad5, *Lefty1* is expressed at very low level, while *Nodal*, *Lefty2* and *Pitx2* are expressed bilaterally, suggesting BMP signaling is required for normal L–R patterning since Smad5 is specific for BMP signaling. However, because *Smad5* is expressed ubiquitously, how directly BMP signaling through Smad5 affect the process of L–R determination is yet to be determined [131].

4.3.3. BMP4 as an important factor for left sidedness

Analyses of tetraploid chimeras using *Bmp4* null ES cells as well as *Bmp4* mutant embryos revealed that *Bmp4* expression is required for node morphogenesis and for L–R patterning [41]. In *Bmp4* null mutant embryos, *Nodal* expression in the node is severely reduced and no expression was observed in the left LPM. Furthermore, the *Bmp4* mutants show an abnormal flat shape of the node in contrast to the concave structure of the node in the wild-type embryos. In the tetraploid chimeras, wild-type extra-embryonic ectoderm can supply BMP4, which restores node morphology. These tetraploid chimeric embryos show no evidence of heart looping (mesocardia) and reduction of *Nodal* expression in the node and LPM. These results suggest that BMP4 signaling is critical to establish left sidedness. However, because *Bmp4* expression is restricted in the extraembryonic mesoderm and primitive streak, but not in the node at presomite stage these defects in the chimeras seem to be caused indirectly. Application of Noggin to cultured embryos at the early somite stages abolishes *Nodal* expression only in the LPM but not in the node, suggesting that BMP signaling by BMP4 in the LPM is required for the induction of *Nodal* in the LPM [41]. Thus, BMP4 plays different roles from other BMP signaling through Smad5 to contribute for establishing left sidedness.

4.3.4. BMP signaling through ACVRI as a critical factor for right-sidedness

Recently, ACVRI (ALK2), a type I receptor for BMPs, is also shown to play important roles in L–R patterning in the mouse. As mentioned above, *AcvrI* null embryos die during gastrulation of which severe phenotype is rescued by supply of wild-type extraembryonic tissue [50,54]. Chimeric embryos using *AcvrI* null ES cells show laterality defects including embryonic turning and heart looping depending on their contributions [53]. *AcvrI* expression is highly confined in the visceral endoderm at E6.5 [51,52] and, later in the node and its derivatives, notochord at E8.0 [52,53]. In the chimeras, ES cells have no ability to contribute to the visceral endoderm. Therefore, the laterality defects of *AcvrI* chimeras seem to be caused by the lack of BMP signaling through ACVRI in the node and notochord. Molecular marker analysis revealed that, like *Smad5* mutant embryos, *AcvrI* chimeras show the significant reduction of *Lefty1* expression in the notochord and bilateral expression of *Nodal*, *Lefty2* and *Pitx2* in the LPM, indicating that the loss of ACVRI signaling leads to left-isomerism. However, distinguishably from *Smad5* mutants, asymmetric expression pattern of *Nodal* in the node is also perturbed in the *AcvrI* chimeras [53]. Although there is no evidence that asymmetric expression of *Nodal* is required for L–R patterning, this finding suggests that BMP signaling through ACVRI in the node regulates *Nodal* expression in the node as an upstream factor. In *Xenopus*, it is reported that ACVRI transduces right-sidedness in a manner that is antagonistic to Vg1-dependent signaling [126]. Together, the requirement of ACVRI signaling for right-sidedness may be conserved beyond species.

It is still unclear how BMP signaling is regulated in the node and which BMPs are ligands for ACVRI in the node. It

has also been reported that Smad5 can transduce BMP6 and BMP7 signals through ACVRI in vitro [132,133]. *Bmp7* is the only known BMP member that is expressed in the node [134], but *Bmp7* null embryos do not show defects in L–R patterning [135,136]. Even if BMP7 is capable of forming heterodimers with *Nodal* [113], its actual meaning in vivo still remains unclear. Interestingly, *Noggin* and *Chordin* double mutant embryos show a randomization of heart looping [24] suggesting BMP signaling in the node might need to be well-tuned by these antagonists for L–R patterning. Identification of BMP ligands for ACVRI is critical in order to elucidate BMP functions in the node. It is also interesting to address whether in the mouse BMP signaling in the node antagonize GDF1/Vg1 signaling as in *Xenopus* [126] or HH signaling [90,137,138] by Shh and Ihh as in the chick [139].

In summary, BMPs are required for normal L–R development both in the node and LPM (Fig. 6). However, it seems to be two distinctive types of BMP signaling, which are opposite for each other. First, BMPs/ACVRI/Smad5 is required at least for the notochord to prevent left-isomerism. Second, BMP4 that presumably signals through BMPRIA is required for the LPM to induce the left-specific genes including *Nodal*. Thus, BMP signaling may contribute to L–R patterning in the tissue- and developmental-stage specific manner. For further analysis, tissue- and stage-specific gene disruption for BMP signaling using a Cre/loxP system would be applied to dissect functions of BMP signaling in each stage and tissue for L–R patterning.

5. Perspective

In this review, we summarized recent achievement in mouse genetics to uncover divergent functions of BMPs during embryonic patterning. Despite the huge amount of new insight gained during last decade, still many questions need to be answered for molecular understanding of the functions of BMPs. Downstream pathways of BMP receptors are more complex than those of other members of TGF-β superfamily, especially during early embryogenesis. Little is know of the target genes of divergent BMP signaling pathways. Chimeric analyses and conditional gene knockout studies clearly show that communication between different tissues and regions play critical BMP-dependent roles to establish the polarities. These suggest that some of the target genes of BMPs would be secreting factors or membrane proteins. Further analyses of these molecules should be awaited for better understanding of the mechanisms how each part of embryos communicates to develop three-dimensional structures.

BMP signaling also plays divergent and important roles during organogenesis [3,9]. Intensive studies in human genetics reveal that mutations in BMP signaling pathways are involved in the pathogenesis of chondrodysplasia, hereditary haemorrhagic telangiectasia, primary pulmonary hypertension, tumorigenesis (Juvenile Polyposis), infertility and cardiac abnormalities [140–147]. Conditional gene knock out of BMPs and their signaling molecules in the mouse, which can escape early lethality, develop similar phenotype as

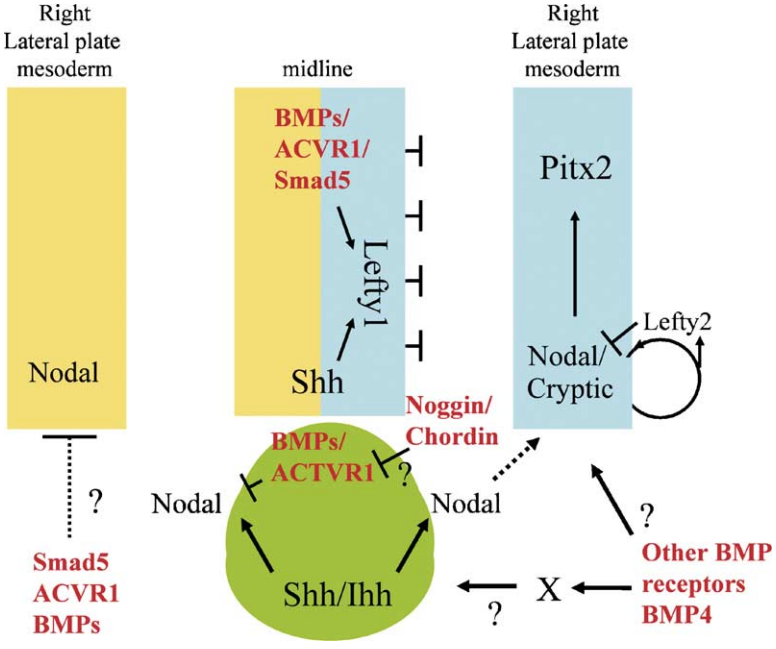

Fig. 6. Model for BMP functions of L–R patterning in the mouse embryo. In the node, BMP signaling through ACVRI modifies *nodal* expression induced by HH signaling. ACVRI signaling in the node may be regulated by BMP antagonists, Noggin and Chordin. BMP4 may also contribute indirectly to nodal induction via an unknown factor. In the midline, BMP signaling through ACVRI/Smad5 is required to induce expression of *lefty1* in the left side. ACVRI/Smad5 signaling acts in parallel with Shh signaling in the midline since both BMP and Shh signaling are required for *Lefty1* expression. In the LPM, Nodal is induced by Nodal signaling from the node. BMP4 also seems to be essential for normal *nodal* expression in the LPM through BMPRIA. It is still unclear whether ACVRI/Smad5 signaling functions to repress the Nodal expression in the right LPM.

these of humans [148–163]. Knowledge obtained from the analyses of the signaling pathways in embryos should also shed light to the pathogenesis of these human diseases.

Acknowledgements

I thank Dr. Hari Reddi for providing me an opportunity to prepare this article. I also thank Drs. Shigeto Miura, Manas K. Ray and Gregory Scott for helpful comments on the manuscript; Kuniko Kishigami, Yoshiko, Kanade H. and Ryoji J. Mishina for their encouragement during the preparation of this article.

References

[1] Urist MR. Bone: formation by autoinduction. Science 1965;150:893–9.
[2] Kingsley DM. The TGF-beta superfamily: new members, new receptors, and new genetic tests of function in different organisms. Genes Dev 1994;8:133–46.
[3] Zhao GQ. Consequences of knocking out BMP signaling in the mouse. Genesis 2003;35:43–56.
[4] Wozney JM. The bone morphogenetic protein family and osteogenesis. Mol Reprod Dev 1992;32:160–7.
[5] Sampath TK, Reddi AH. Dissociative extraction and reconstitution of extracellular matrix components involved in local bone differentiation. Proc Natl Acad Sci USA 1981;78:7599–603.
[6] Reddi AH. Bone and cartilage differentiation. Curr Opin Genet Dev 1994;4:737–44.
[7] Hogan BL. Bone morphogenetic proteins in development. Curr Opin Genet Dev 1996;6:432–8.
[8] Whitman M. Smads and early developmental signaling by the TGFbeta superfamily. Genes Dev 1998;12:2445–62.
[9] Mishina Y. Function of bone morphogenetic protein signaling during mouse development. Front Biosci 2003;8:d855–69.
[10] Ray RP, Arora K, Nusslein-Volhard C, Gelbart WM. The control of cell fate along the dorsal–ventral axis of the Drosophila embryo. Development 1991;113:35–54.
[11] Dale L, Howes G, Price BM, Smith JC. Bone morphogenetic protein 4: a ventralizing factor in early Xenopus development. Development 1992;115:573–85.
[12] Jones CM, Lyons KM, Lapan PM, Wright CV, Hogan BL. DVR-4 (bone morphogenetic protein-4) as a posterior-ventralizing factor in Xenopus mesoderm induction. Development 1992;115:639–47.
[13] Wrana JL, Attisano L, Wieser R, Ventura F, Massague J. Mechanism of activation of the TGF-beta receptor. Nature 1994;370:341–7.
[14] Heldin CH, Miyazono K, ten Dijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. Nature 1997;390:465–71.
[15] Franzen P, ten Dijke P, Ichijo H, et al. Cloning of a TGF beta type I receptor that forms a heteromeric complex with the TGF beta type II receptor. Cell 1993;75:681–92.
[16] Carcamo J, Weis FM, Ventura F, et al. Type I receptors specify growth-inhibitory and transcriptional responses to transforming growth factor beta and activin. Mol Cell Biol 1994;14:3810–21.
[17] Massague J. TGFbeta signaling: receptors, transducers, and Mad proteins. Cell 1996;85:947–50.
[18] de Caestecker M. The transforming growth factor-beta superfamily of receptors. Cytokine Growth Factor Rev 2004;15:1–11.
[19] Giudice G. Conserved cellular and molecular mechanisms in development. Cell Biol Int 2001;25:1081–90.
[20] Sasai Y. Regulation of neural determination by evolutionarily conserved signals: anti-BMP factors and what next? Curr Opin Neurobiol 2001;11:22–6.
[21] Piccolo S, Agius E, Leyns L, et al. The head inducer Cerberus is a multifunctional antagonist of Nodal BMP and Wnt signals. Nature 1999;397:707–10.
[22] Belo JA, Bachiller D, Agius E, et al. Cerberus-like is a secreted BMP and nodal antagonist not essential for mouse development. Genesis 2000;26:265–70.
[23] Anderson RM, Lawrence AR, Stottmann RW, Bachiller D, Klingensmith J. Chordin and noggin promote organizing centers of forebrain development in the mouse. Development 2002;129:4975–87.
[24] Bachiller D, Klingensmith J, Kemp C, et al. The organizer factors Chordin and Noggin are required for mouse forebrain development. Nature 2000;403:658–61.
[25] Bachiller D, Klingensmith J, Shneyder N, et al. The role of chordin/Bmp signals in mammalian pharyngeal development and DiGeorge syndrome. Development 2003;130:3567–78.
[26] Shawlot W, Min Deng J, Wakamiya M, Behringer RR. The cerberus-related gene, Cerr1, is not essential for mouse head formation. Genesis 2000;26:253–8.
[27] Tam PP, Behringer RR. Mouse gastrulation: the formation of a mammalian body plan. Mech Dev 1997;68:3–25.
[28] Beddington RS, Robertson EJ. Axis development and early asymmetry in mammals. Cell 1999;96:195–209.
[29] Behringer RR, Wakamiya M, Tsang TE, Tam PP. A flattened mouse embryo: leveling the playing field. Genesis 2000;28:23–30.
[30] Rossant J, Tam PP. Emerging asymmetry and embryonic patterning in early mouse development. Dev Cell 2004;7:155–64.
[31] Lu CC, Brennan J, Robertson EJ. From fertilization to gastrulation: axis formation in the mouse embryo. Curr Opin Genet Dev 2001;11:384–92.
[32] Thomas PQ, Brown A, Beddington RS. Hex: a homeobox gene revealing peri-implantation asymmetry in the mouse embryo and an early transient marker of endothelial cell precursors. Development 1998;125:85–94.
[33] Rivera-Perez JA, Mager J, Magnuson T. Dynamic morphogenetic events characterize the mouse visceral endoderm. Dev Biol 2003;261:470–87.
[34] Srinivas S, Rodriguez T, Clements M, Smith JC, Beddington RS. Active cell migration drives the unilateral movements of the anterior visceral endoderm. Development 2004;131:1157–64.
[35] Nagy A, Rossant J. Chimaeras and mosaics for dissecting complex mutant phenotypes. Int J Dev Biol 2001;45:577–82.
[36] Nagy A. Cre recombinase: the universal reagent for genome tailoring. Genesis 2000;26:99–109.
[37] Winnier G, Blessing M, Labosky PA, Hogan BL. Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. Genes Dev 1995;9:2105–16.
[38] Lawson KA, Dunn NR, Roelen BA, et al. Bmp4 is required for the generation of primordial germ cells in the mouse embryo. Genes Dev 1999;13:424–36.
[39] Ying Y, Liu XM, Marble A, Lawson KA, Zhao GQ. Requirement of Bmp8b for the generation of primordial germ cells in the mouse. Mol Endocrinol 2000;14:1053–63.
[40] Ying Y, Zhao GQ. Cooperation of endoderm-derived BMP2 and extraembryonic ectoderm-derived BMP4 in primordial germ cell generation in the mouse. Dev Biol 2001;232:484–92.
[41] Fujiwara T, Dehart DB, Sulik KK, Hogan BL. Distinct requirements for extra-embryonic and embryonic bone morphogenetic protein 4 in the formation of the node and primitive streak and coordination of left–right asymmetry in the mouse. Development 2002;129:4685–96.
[42] Nagy A, Rossant J, Nagy R, Abramow-Newerly W, Roder JC. Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. Proc Natl Acad Sci USA 1993;90:8424–8.

[43] Zhang H, Bradley A. Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. Development 1996;122:2977–86.
[44] Mishina Y, Suzuki A, Ueno N, Behringer RR. Bmpr encodes a type I bone morphogenetic protein receptor that is essential for gastrulation during mouse embryogenesis. Genes Dev 1995;9:3027–37.
[45] Mishina Y, Hanks MC, Miura S, Tallquist MD, Behringer RR. Generation of Bmpr/Alk3 conditional knockout mice. Genesis 2002;32:69–72.
[46] Tallquist MD, Soriano P. Epiblast-restricted Cre expression in MORE mice: a tool to distinguish embryonic vs. extra-embryonic gene function. Genesis 2000;26:113–5.
[47] Davis S, Miura S, Hill C, Mishina Y, Klingensmith J. BMP receptor IA is required in the mammalian embryo for endodermal morphogenesis and ectodermal patterning. Dev Biol 2004;270:47–63.
[48] Qi X, Li TG, Hao J, et al. BMP4 supports self-renewal of embryonic stem cells by inhibiting mitogen-activated protein kinase pathways. Proc Natl Acad Sci USA 2004;101:6027–32.
[49] Ying QL, Nichols J, Chambers I, Smith A. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. Cell 2003;115:281–92.
[50] Gu Z, Reynolds EM, Song J, et al. The type I serine/threonine kinase receptor ActRIA (ALK2) is required for gastrulation of the mouse embryo. Development 1999;126:2551–61.
[51] Roelen BA, Lin HY, Knezevic V, Freund E, Mummery CL. Expression of TGF-beta s and their receptors during implantation and organogenesis of the mouse embryo. Dev Biol 1994;166:716–28.
[52] Yoshikawa SI, Aota S, Shirayoshi Y, Okazaki K. The ActR-I activin receptor protein is expressed in notochord, lens placode and pituitary primordium cells in the mouse embryo. Mech Dev 2000;91:439–44.
[53] Kishigami S, Yoshikawa S, Castranio T, Okazaki K, Furuta Y, Mishina Y. BMP signaling through ACVRI is required for left–right patterning in the early mouse embryo. Dev Biol 2004;276:185–93.
[54] Mishina Y, Crombie R, Bradley A, Behringer RR. Multiple roles for activin-like kinase-2 signaling during mouse embryogenesis. Dev Biol 1999;213:314–26.
[55] de Sousa Lopes SM, Roelen BA, Monteiro RM, et al. BMP signaling mediated by ALK2 in the visceral endoderm is necessary for the generation of primordial germ cells in the mouse embryo. Genes Dev 2004;18:1838–49.
[56] Ying Y, Qi X, Zhao GQ. Induction of primordial germ cells from murine epiblasts by synergistic action of BMP4 and BMP8B signaling pathways. Proc Natl Acad Sci USA 2001;98:7858–62.
[57] Kaartinen V, Nagy A. Removal of the floxed neo gene from a conditional knockout allele by the adenoviral Cre recombinase in vivo. Genesis 2001;31:126–9.
[58] Beppu H, Kawabata M, Hamamoto T, et al. BMP type II receptor is required for gastrulation and early development of mouse embryos. Dev Biol 2000;221:249–58.
[59] Tremblay KD, Dunn NR, Robertson EJ. Mouse embryos lacking Smad1 signals display defects in extra-embryonic tissues and germ cell formation. Development 2001;128:3609–21.
[60] Lechleider RJ, Ryan JL, Garrett L, et al. Targeted mutagenesis of Smad1 reveals an essential role in chorioallantoic fusion. Dev Biol 2001;240:157–67.
[61] Reissmann E, Jornvall H, Blokzijl A, et al. The orphan receptor ALK7 and the Activin receptor ALK4 mediate signaling by Nodal proteins during vertebrate development. Genes Dev 2001;15:2010–22.
[62] Zhou X, Sasaki H, Lowe L, Hogan BL, Kuehn MR. Nodal is a novel TGF-beta-like gene expressed in the mouse node during gastrulation. Nature 1993;361:543–7.
[63] Conlon FL, Lyons KM, Takaesu N, et al. A primary requirement for nodal in the formation and maintenance of the primitive streak in the mouse. Development 1994;120:1919–28.
[64] Brennan J, Lu CC, Norris DP, Rodriguez TA, Beddington RS, Robertson EJ. Nodal signalling in the epiblast patterns the early mouse embryo. Nature 2001;411:965–9.
[65] Norris DP, Robertson EJ. Asymmetric and node-specific nodal expression patterns are controlled by two distinct cis-acting regulatory elements. Genes Dev 1999;13:1575–88.
[66] Varlet I, Collignon J, Robertson EJ. nodal expression in the primitive endoderm is required for specification of the anterior axis during mouse gastrulation. Development 1997;124:1033–44.
[67] Meno C, Gritsman K, Ohishi S, et al. Mouse Lefty2 and zebrafish antivin are feedback inhibitors of nodal signaling during vertebrate gastrulation. Mol Cell 1999;4:287–98.
[68] Perea-Gomez A, Vella FD, Shawlot W, et al. Nodal antagonists in the anterior visceral endoderm prevent the formation of multiple primitive streaks. Dev Cell 2002;3:745–56.
[69] Yamamoto M, Saijoh Y, Perea-Gomez A, et al. Nodal antagonists regulate formation of the anteroposterior axis of the mouse embryo. Nature 2004;428:387–92.
[70] Furuta Y, Hogan BL. BMP4 is essential for lens induction in the mouse embryo. Genes Dev 1998;12:3764–75.
[71] Fujiwara T, Dunn NR, Hogan BL. Bone morphogenetic protein 4 in the extraembryonic mesoderm is required for allantois development and the localization and survival of primordial germ cells in the mouse. Proc Natl Acad Sci USA 2001;98:13739–44.
[72] Shawlot W, Wakamiya M, Kwan KM, Kania A, Jessell TM, Behringer RR. Lim1 is required in both primitive streak-derived tissues and visceral endoderm for head formation in the mouse. Development 1999;126:4925–32.
[73] Dewulf N, Verschueren K, Lonnoy O, et al. Distinct spatial and temporal expression patterns of two type I receptors for bone morphogenetic proteins during mouse embryogenesis. Endocrinology 1995;136:2652–3663.
[74] Baur ST, Mai JJ, Dymecki SM. Combinatorial signaling through BMP receptor IB and GDF5: shaping of the distal mouse limb and the genetics of distal limb diversity. Development 2000;127:605–19.
[75] Yi SE, Daluiski A, Pederson R, Rosen V, Lyons KM. The type I BMP receptor BMPRIB is required for chondrogenesis in the mouse limb. Development 2000;127:621–30.
[76] Norris DP, Brennan J, Bikoff EK, Robertson EJ. The Foxh1-dependent autoregulatory enhancer controls the level of Nodal signals in the mouse embryo. Development 2002;129:3455–68.
[77] Lu CC, Robertson EJ. Multiple roles for Nodal in the epiblast of the mouse embryo in the establishment of anterior–posterior patterning. Dev Biol 2004;273:149–59.
[78] Nomura M, Li E. Smad2 role in mesoderm formation, left–right patterning and craniofacial development. Nature 1998;393: 786–90.
[79] Waldrip WR, Bikoff EK, Hoodless PA, Wrana JL, Robertson EJ. Smad2 signaling in extraembryonic tissues determines anterior–posterior polarity of the early mouse embryo. Cell 1998;92: 797–808.
[80] Tremblay KD, Hoodless PA, Bikoff EK, Robertson EJ. Formation of the definitive endoderm in mouse is a Smad2-dependent process. Development 2000;127:3079–90.
[81] Vincent SD, Dunn NR, Hayashi S, Norris DP, Robertson EJ. Cell fate decisions within the mouse organizer are governed by graded Nodal signals. Genes Dev 2003;17:1646–62.
[82] Zhu Y, Richardson JA, Parada LF, Graff JM. Smad3 mutant mice develop metastatic colorectal cancer. Cell 1998;94:703–14.
[83] Datto MB, Frederick JP, Pan L, Borton AJ, Zhuang Y, Wang XF. Targeted disruption of Smad3 reveals an essential role in transforming growth factor beta-mediated signal transduction. Mol Cell Biol 1999;19:2495–504.
[84] Yang X, Letterio JJ, Lechleider RJ, et al. Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. EMBO J 1999;18:1280–91.
[85] Dunn NR, Vincent SD, Oxburgh L, Robertson EJ, Bikoff EK. Combinatorial activities of Smad2 and Smad3 regulate mesoderm formation and patterning in the mouse embryo. Development 2004;131:1717–28.

[86] Sirard C, de la Pompa JL, Elia A, et al. The tumor suppressor gene Smad4/Dpc4 is required for gastrulation and later for anterior development of the mouse embryo. Genes Dev 1998;12:107–19.
[87] Yang X, Li C, Xu X, Deng C. The tumor suppressor SMAD4/DPC4 is essential for epiblast proliferation and mesoderm induction in mice. Proc Natl Acad Sci USA 1998;95:3667–72.
[88] Chu GC, Dunn NR, Anderson DC, Oxburgh L, Robertson EJ. Differential requirements for Smad4 in TGFbeta-dependent patterning of the early mouse embryo. Development 2004;131:3501–12.
[89] Hamada H, Meno C, Watanabe D, Saijoh Y. Establishment of vertebrate left–right asymmetry. Nat Rev Genet 2002;3:103–13.
[90] Meyers EN, Martin GR. Differences in left–right axis pathways in mouse and chick: functions of FGF8 and SHH. Science 1999;285: 403–6.
[91] Wright CV. Mechanisms of left–right asymmetry: what's right and what's left? Dev Cell 2001;1:179–86.
[92] Capdevila J, Vogan KJ, Tabin CJ, Izpisua Belmonte JC. Mechanisms of left–right determination in vertebrates. Cell 2000;101:9–21.
[93] Palmer AR. Symmetry breaking and the evolution of development. Science 2004;306:828–33.
[94] Logan M, Pagan-Westphal SM, Smith DM, Paganessi L, Tabin CJ. The transcription factor Pitx2 mediates situs-specific morphogenesis in response to left–right asymmetric signals. Cell 1998;94:307–17.
[95] Piedra ME, Icardo JM, Albajar M, Rodriguez-Rey JC, Ros MA. Pitx2 participates in the late phase of the pathway controlling left–right asymmetry. Cell 1998;94:319–24.
[96] Yoshioka H, Meno C, Koshiba K, et al. Pitx2, a bicoid-type homeobox gene, is involved in a lefty-signaling pathway in determination of left–right asymmetry. Cell 1998;94:299–305.
[97] Fujinaga M. Development of sidedness of asymmetric body structures in vertebrates. Int J Dev Biol 1997;41:153–86.
[98] Nonaka S, Tanaka Y, Okada Y, et al. Randomization of left–right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. Cell 1998;95:829–37.
[99] Essner JJ, Vogan KJ, Wagner MK, Tabin CJ, Yost HJ, Brueckner M. Conserved function for embryonic nodal cilia. Nature 2002;418:37–8.
[100] Okada Y, Nonaka S, Tanaka Y, Saijoh Y, Hamada H, Hirokawa N. Abnormal nodal flow precedes situs inversus in iv and inv mice. Mol Cell 1999;4:459–68.
[101] Nonaka S, Shiratori H, Saijoh Y, Hamada H. Determination of left–right patterning of the mouse embryo by artificial nodal flow. Nature 2002;418:96–9.
[102] Collignon J, Varlet I, Robertson EJ. Relationship between asymmetric nodal expression and the direction of embryonic turning. Nature 1996;381:155–8.
[103] Lowe LA, Supp DM, Sampath K, et al. Conserved left–right asymmetry of nodal expression and alterations in murine situs inversus. Nature 1996;381:158–61.
[104] Lowe LA, Yamada S, Kuehn MR. Genetic dissection of nodal function in patterning the mouse embryo. Development 2001;128:1831–43.
[105] Brennan J, Norris DP, Robertson EJ. Nodal activity in the node governs left–right asymmetry. Genes Dev 2002;16:2339–44.
[106] Saijoh Y, Oki S, Ohishi S, Hamada H. Left–right patterning of the mouse lateral plate requires nodal produced in the node. Dev Biol 2003;256:160–72.
[107] Oh SP, Li E. The signaling pathway mediated by the type IIB activin receptor controls axial patterning and lateral asymmetry in the mouse. Genes Dev 1997;11:1812–26.
[108] Song J, Oh SP, Schrewe H, et al. The type II activin receptors are essential for egg cylinder growth, gastrulation, and rostral head development in mice. Dev Biol 1999;213:157–69.
[109] Oh SP, Li E. Gene-dosage-sensitive genetic interactions between inversus viscerum (iv), nodal, and activin type IIB receptor (ActRIIB) genes in asymmetrical patterning of the visceral organs along the left–right axis. Dev Dyn 2002;224:279–90.
[110] Raya A, Belmonte JC. Sequential transfer of left–right information during vertebrate embryo development. Curr Opin Genet Dev 2004;14:575–81.
[111] Rankin CT, Bunton T, Lawler AM, Lee SJ. Regulation of left–right patterning in mice by growth/differentiation factor-1. Nat Genet 2000;24:262–5.
[112] Wall NA, Craig EJ, Labosky PA, Kessler DS. Mesendoderm induction and reversal of left–right pattern by mouse Gdf1, a Vg1-related gene. Dev Biol 2000;227:495–509.
[113] Yeo C, Whitman M. Nodal signals to Smads through cripto-dependent and cripto-independent mechanisms. Mol Cell 2001;7:949–57.
[114] Yan YT, Gritsman K, Ding J, et al. Conserved requirement for EGF-CFC genes in vertebrate left–right axis formation. Genes Dev 1999;13:2527–37.
[115] Gaio U, Schweickert A, Fischer A, et al. A role of the cryptic gene in the correct establishment of the left–right axis. Curr Biol 1999;9:1339–42.
[116] Meno C, Shimono A, Saijoh Y, et al. lefty-1 is required for left–right determination as a regulator of lefty-2 and nodal. Cell 1998;94:287–97.
[117] Sakuma R, Ohnishi Yi Y, Meno C, et al. Inhibition of Nodal signalling by Lefty mediated through interaction with common receptors and efficient diffusion. Genes Cells 2002;7:401–12.
[118] Yamamoto M, Mine N, Mochida K, et al. Nodal signaling induces the midline barrier by activating Nodal expression in the lateral plate. Development 2003;130:1795–804.
[119] Branford WW, Yost HJ. Nodal signaling: cryptic lefty mechanism of antagonism decoded. Curr Biol 2004;14:341–3.
[120] Chen C, Shen MM. Two modes by which Lefty proteins inhibit nodal signaling. Curr Biol 2004;14:618–24.
[121] Cheng SK, Olale F, Brivanlou AH, Schier AF. Lefty blocks a subset of TGFbeta signals by antagonizing EGF-CFC coreceptors. PLoS Biol 2004;2:30.
[122] Meno C, Takeuchi J, Sakuma R, et al. Diffusion of nodal signaling activity in the absence of the feedback inhibitor Lefty2. Dev Cell 2001;1:127–38.
[123] Meno C, Ito Y, Saijoh Y, et al. Two closely-related left–right asymmetrically expressed genes, lefty-1 and lefty-2: their distinct expression domains, chromosomal linkage and direct neuralizing activity in Xenopus embryos. Genes Cells 1997;2:513–24.
[124] Branford WW, Essner JJ, Yost HJ. Regulation of gut and heart left–right asymmetry by context-dependent interactions between Xenopus lefty and BMP4 signaling. Dev Biol 2000;223:291–306.
[125] Ulloa L, Tabibzadeh S. Lefty inhibits receptor-regulated Smad phosphorylation induced by the activated transforming growth factor-beta receptor. J Biol Chem 2001;276:21397–404.
[126] Ramsdell AF, Yost HJ. Cardiac looping and the vertebrate left–right axis: antagonism of left-sided Vg1 activity by a right-sided ALK2-dependent BMP pathway. Development 1999;126:5195–205.
[127] Rodriguez Esteban C, Capdevila J, Economides AN, Pascual J, Ortiz A, Izpisua Belmonte JC. The novel Cer-like protein Caronte mediates the establishment of embryonic left–right asymmetry. Nature 1999;401:243–51.
[128] Yokouchi Y, Vogan KJ, Pearse 2nd RV, Tabin CJ. Antagonistic signaling by Caronte, a novel Cerberus-related gene, establishes left–right asymmetric gene expression. Cell 1999;98:573–83.
[129] Piedra ME, Ros MA. BMP signaling positively regulates Nodal expression during left right specification in the chick embryo. Development 2002;129:3431–40.
[130] Schlange T, Arnold HH, Brand T. BMP2 is a positive regulator of Nodal signaling during left–right axis formation in the chicken embryo. Development 2002;129:3421–9.
[131] Chang H, Zwijsen A, Vogel H, Huylebroeck D, Matzuk MM. Smad5 is essential for left–right asymmetry in mice. Dev Biol 2000;219:71–8.
[132] Ebisawa T, Tada K, Kitajima I, et al. Characterization of bone morphogenetic protein-6 signaling pathways in osteoblast differentiation. J Cell Sci 1999;112(Pt 20):3519–27.

[133] Macias-Silva M, Hoodless PA, Tang SJ, Buchwald M, Wrana JL. Specific activation of Smad1 signaling pathways by the BMP7 type I receptor, ALK2. J Biol Chem 1998;273:25628–36.
[134] Solloway MJ, Robertson EJ. Early embryonic lethality in Bmp5; Bmp7 double mutant mice suggests functional redundancy within the 60A subgroup. Development 1999;126:1753–68.
[135] Dudley AT, Lyons KM, Robertson EJ. A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. Genes Dev 1995;9:2795–807.
[136] Luo G, Hofmann C, Bronckers AL, Sohocki M, Bradley A, Karsenty G. BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. Genes Dev 1995;9:2808–20.
[137] Tsukui T, Capdevila J, Tamura K, et al. Multiple left–right asymmetry defects in Shh(−/−) mutant mice unveil a convergence of the shh and retinoic acid pathways in the control of Lefty-1. Proc Natl Acad Sci USA 1999;96:11376–81.
[138] Zhang XM, Ramalho-Santos M, McMahon AP. Smoothened mutants reveal redundant roles for Shh and Ihh signaling including regulation of L/R symmetry by the mouse node. Cell 2001;106:781–92.
[139] Monsoro-Burq A, Le Douarin NM. BMP4 plays a key role in left–right patterning in chick embryos by maintaining Sonic Hedgehog asymmetry. Mol Cell 2001;7:789–99.
[140] Thomas JT, Lin K, Nandedkar M, Camargo M, Cervenka J, Luyten FP. A human chondrodysplasia due to a mutation in a TGF-beta superfamily member. Nat Genet 1996;12:315–7.
[141] Thomas JT, Kilpatrick MW, Lin K, et al. Disruption of human limb morphogenesis by a dominant negative mutation in CDMP1. Nat Genet 1997;17:58–64.
[142] Johnson DW, Berg JN, Baldwin MA, et al. Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. Nat Genet 1996;13:189–95.
[143] Lane KB, Machado RD, Pauciulo MW, et al. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. The International PPH Consortium. Nat Genet 2000;26:81–4.
[144] Zhou XP, Woodford-Richens K, Lehtonen R, et al. Germline mutations in BMPR1A/ALK3 cause a subset of cases of juvenile polyposis syndrome and of Cowden and Bannayan–Riley–Ruvalcaba syndromes. Am J Hum Genet 2001;69:704–11.
[145] Howe JR, Bair JL, Sayed MG, et al. Germline mutations of the gene encoding bone morphogenetic protein receptor 1A in juvenile polyposis. Nat Genet 2001;28:184–7.
[146] Imbeaud S, Faure E, Lamarre I, et al. Insensitivity to anti-mullerian hormone due to a mutation in the human anti-mullerian hormone receptor. Nat Genet 1995;11:382–8.
[147] Pashmforoush M, Lu JT, Chen H, et al. Nkx2-5 pathways and congenital heart disease; loss of ventricular myocyte lineage specification leads to progressive cardiomyopathy and complete heart block. Cell 2004;117:373–86.
[148] Rountree RB, Schoor M, Chen H, et al. BMP receptor signaling is required for postnatal maintenance of articular cartilage. PLoS Biol 2004;2:e355.
[149] Mishina Y, Starbuck MW, Gentile MA, et al. Bone morphogenetic protein type IA receptor signaling regulates postnatal osteoblast function and bone remodeling. J Biol Chem 2004;279:27560–6.
[150] Oh SP, Seki T, Goss KA, et al. Activin receptor-like kinase 1 modulates transforming growth factor-beta 1 signaling in the regulation of angiogenesis. Proc Natl Acad Sci USA 2000;97:2626–31.
[151] Zhang J, Niu C, Ye L, et al. Identification of the haematopoietic stem cell niche and control of the niche size. Nature 2003;425:836–41.
[152] He XC, Zhang J, Tong WG, et al. BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-beta-catenin signaling. Nat Genet 2004;36:1117–21.
[153] Jamin SP, Arango NA, Mishina Y, Hanks MC, Behringer RR. Requirement of Bmpr1a for Mullerian duct regression during male sexual development. Nat Genet 2002;32:408–10.
[154] Yuhki M, Yamada M, Kawano M, et al. BMPR1A signaling is necessary for hair follicle cycling and hair shaft differentiation in mice. Development 2004;131:1825–33.
[155] Andl T, Ahn K, Kairo A, et al. Epithelial Bmpr1a regulates differentiation and proliferation in postnatal hair follicles and is essential for tooth development. Development 2004;131:2257–68.
[156] Ming Kwan K, Li AG, Wang XJ, Wurst W, Behringer RR. Essential roles of BMPR-IA signaling in differentiation and growth of hair follicles and in skin tumorigenesis. Genesis 2004;39:10–25.
[157] Kobielak K, Pasolli HA, Alonso L, Polak L, Fuchs E. Defining BMP functions in the hair follicle by conditional ablation of BMP receptor IA. J Cell Biol 2003;163:609–23.
[158] Gaussin V, Van de Putte T, Mishina Y, et al. Endocardial cushion and myocardial defects after cardiac myocyte-specific conditional deletion of the bone morphogenetic protein receptor ALK3. Proc Natl Acad Sci USA 2002;99:2878–83.
[159] Jiao K, Kulessa H, Tompkins K, et al. An essential role of Bmp4 in the atrioventricular septation of the mouse heart. Genes Dev 2003;17:2362–7.
[160] Oh H, Chi X, Bradfute SB, et al. Cardiac muscle plasticity in adult and embryo by heart-derived progenitor cells. Ann N Y Acad Sci 2004;1015:182–9.
[161] Kaartinen V, Dudas M, Nagy A, Sridurongrit S, Lu MM, Epstein JA. Cardiac outflow tract defects in mice lacking ALK2 in neural crest cells. Development 2004;131:3481–90.
[162] Dudas M, Sridurongrit S, Nagy A, Okazaki K, Kaartinen V. Craniofacial defects in mice lacking BMP type I receptor Alk2 in neural crest cells. Mech Dev 2004;121:173–82.
[163] Chen H, Shi S, Acosta L, et al. BMP10 is essential for maintaining cardiac growth during murine cardiogenesis. Development 2004; 131:2219–31.