The transcription factor FoxH1 (FAST) mediates Nodal signaling during anterior-posterior patterning and node formation in the mouse

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FoxH1 (FAST) is a transcription factor that mediates signaling by transforming growth factor–β, Activin, and Nodal. The role of FoxH1 in development has now been investigated by the generation and analysis of FoxH1-deficient (FoxH1−/−) mice. The FoxH1−/− embryos showed various patterning defects that recapitulate most of the defects induced by the loss of Nodal signaling. A substantial proportion of FoxH1−/− embryos failed to orient the anterior-posterior (A-P) axis correctly, as do mice lacking Cripto, a coreceptor for Nodal. In less severely affected FoxH1−/− embryos, A-P polarity was established, but the primitive streak failed to elongate, resulting in the lack of a definitive node and its derivatives. Heterozygosity for nodal renders the FoxH1−/− phenotype more severe, indicative of a genetic interaction between FoxH1 and nodal. The expression of FoxH1 in the primitive endoderm rescued the A-P patterning defects, but not the midline defects, of FoxH1−/− mice. These results indicate that a Nodal-FoxH1 signaling pathway plays a central role in A-P patterning and node formation in the mouse.

[Key Words: anterior-posterior patterning; FoxH1; gastrulation; Nodal; node]

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Factors related to transforming growth factor–β (TGF-β) control multiple aspects of early development in vertebrates. One such factor, Nodal [Zhou et al. 1993], is a potent signaling molecule that is required for specification of the anterior-posterior [A-P] axis, formation of the primitive streak, and left-right patterning (Schier and Shen 2000). In general, TGF-β-related factors initiate intracellular signaling by interacting with type I and type II receptors on the cell surface, which in turn activate intracellular effectors known as Smad proteins [Massague 1998]. The activated Smad proteins then translocate to the nucleus, where they interact with transcription factors and thereby regulate the expression of target genes. Although the Nodal signaling pathway remains to be fully characterized, genetic evidence suggests that ALK4 [ActRIB] functions as a type I receptor and that ActRIIA and ActRIIB serve as type II receptors in this pathway. Nodal activity is modulated by extracellular cofactors that belong to the EGF-CFC family of proteins [Gritsman et al. 1999], as well as by inhibitors that belong to the Lefty [Meno et al. 1999] and Cerberus families [Piccolo et al. 1999]. Intracellular effectors of Nodal signaling most likely include Smad2, Smad3, and Smad4; consistent with this notion, Smad2 mutant mice exhibit early patterning defects that can be explained by a lack of Nodal signaling in the extraembryonic endoderm [Nomura and Li 1998; Waldrip et al. 1998; Heyer et al. 1999].

The transcription factor FoxH1 (FAST) also mediates Nodal signaling. This protein is a winged-helix transcription factor that was initially identified in Xenopus as a transducer of Activin signaling [Chen et al. 1996]. Thus, FAST-1 [the FoxH1 ortholog in Xenopus] forms a complex with Smad2 and Smad4 in response to Activin and activates a set of genes that includes Mix1 and goosecoid [Chen et al. 1997; Watanabe and Whitman 1999]. However, recent studies have revealed that, in cultured mammalian cells or frog animal caps, this transcription factor also mediates signaling by TGF-β [Labbe et al. 1998; Zhou et al. 1998; Liu et al. 1999] and Nodal [Saijoh et al. 2000]. In particular, FoxH1 appears to induce asymmetric expression of lefty2 in response to Nodal signaling [Osada et al. 2000; Saijoh et al. 2000]. In the mouse, FoxH1 is expressed in early embryos (until the early somite stage) but is rapidly down-regulated as nodal expression disappears [Weisberg et al. 1998; Saijoh et al. 2000].
The expression patterns of nodal and FoxH1 thus appear to overlap with each other. Together, these observations have implicated FoxH1 in Nodal signaling.

Both Smad2 and Smad3 interact with a large number of transcription factors, including TCF, NF-κB, Mix, and Gli (Whitman 1998). It has therefore remained possible that Nodal signaling is mediated in vivo by the interaction of Smad proteins with transcription factors other than FoxH1. Different transcription factors also may mediate Nodal signaling in different cell types. To determine the role of FoxH1 in Nodal signaling, we have therefore generated and characterized mutant mice that lack this transcription factor. The mutant embryos were shown to die early during embryonic development and to show various patterning defects that can be explained by a deficiency in Nodal signaling. Our results indicate that FoxH1 indeed mediates Nodal signaling during A-P patterning and node formation.

Results

Embryonic mortality of FoxH1−/− mice

To investigate the role of FoxH1 in development, we generated mutant mice that lack this transcription factor. The FoxH1 gene is closely linked to KIF-C2 in the reverse orientation; indeed, these two genes share a common 3’ untranslated region [Liu et al. 1999]. We therefore determined to remove exon 1 of FoxH1, leaving KIF-C2 intact [Fig. 1A]. A targeting vector was designed to insert a Frt-flanked neo gene and a loxP site into the 5’ upstream region of FoxH1 and to insert an additional loxP site into intron 1. Two embryonic stem (ES) cell lines (F94, F128) that had undergone homologous recombination were obtained [Fig. 1B], and the F94 ES cells were used to generate chimeric mice. Exon 1 of FoxH1 and the neo gene were excised by crossing the chimeric animals [Fig. 1C]. To generate a flox (flanked by loxP) allele (FoxH1flox), we treated F128 ES cells with a Flp expression vector. One resulting clone, F128-10, from which neo had been correctly excised, was used to generate mice with the FoxH1flox allele [Fig. 1C]. Most of the analyses described in the present study focused on FoxH1−/− mice.

Both FoxH1+/− mice and FoxH1flox/+ heterozygous mice appeared normal and fertile. Genotype analysis at weaning of progeny produced from intercrosses of FoxH1+/− heterozygotes revealed the absence of homozygous mutant animals, indicating that FoxH1−/− mutants die during embryonic development. The FoxH1− allele...
lacking the exon 1 is probably a null allele because FoxH1-related RNA was not detected in FoxH1−/− embryos by in situ hybridization with a full-length FoxH1 anti-sense probe (data not shown) or by reverse transcription-polymerase chain reaction (RT-PCR; Fig. 1D).

**Variable pattern defects in FoxH1−/− embryos**

To characterize the embryonic lethality of the homozygous FoxH1 null allele, we analyzed between embryonic day 7.0 (E7.0) and E11.5 litters produced from heterozygote intercrosses. FoxH1−/− embryos showed a variable phenotype that could be classified into three types. Embryos with the type I (least severe) phenotype show marked axial defects, lacking a definitive node and notochord. Embryos with the type II (intermediate) phenotype completely lack anterior structures but possess posterior structures with midline defects. Embryos with the type III (most severe) phenotype lack structures derived from the embryo proper as a result of A-P patterning defects; they thus resemble Cripto mutant embryos (Ding et al. 1998).

The three types of FoxH1−/− embryos were distinguishable morphologically at E8.5 (Fig. 2). Type I embryos (65/
152 of FoxH1−/− embryos, 43%) showed a single, narrow-fused head structure, or pinhead-like morphology (Fig. 2F,G). In transverse sections, the primitive streak was detected (Fig. 2H), but midline structures such as the node, prechordal plate, notochordal plate–notochord, and floor plate were missing (Fig. 2I,J). The neural plate was unfolded and markedly thickened (Fig. 2I,J), and ectopic ingresson of mesoderm was apparent between the neural plate and the primitive endoderm (Fig. 2J).

Somites had formed, but they were fewer in number than in wild-type embryos and were fused in the midline. Type II mutant embryos (31/152 of FoxH1−/− embryos, 20%) showed severe anterior truncation; they completely lacked anterior structures, such as the head fold, and instead manifested accumulation of cells in the distal region (occasionally, a beating heart–like structure was observed). Extraembryonic tissues such as the amnion and allantois, which were absent in type III embryos, were properly formed (Fig. 2K).

The posterior portion of the embryo was formed but showed severe midline defects. Thus, several somites had formed, but they were fused across the midline (Fig. 2K–M). Type III embryos (52/152 of FoxH1−/− embryos, 34%) showed no signs of development of the embryo proper and showed a balloon-like morphology at this time (Fig. 2N). The embryo proper was recognized as a small cell mass located outside the yolk sac. In contrast, the yolk sac appeared relatively normal; it consisted of endodermal and mesothelial layers and contained blood islands. However, other components of extraembryonic tissue, such as the chorion, amnion, and allantois, were not observed, suggesting that the A-P axis was not properly formed. Rare embryos (4/152 of FoxH1−/− embryos, 3%) showed relatively normal extraembryonic components with no embryo proper (Fig. 2O–Q) and therefore resembled Smad2 mutant embryos (Waldrip et al. 1998).

Three types of FoxH1−/− embryos were also distinguishable on the basis of their morphology at E7.5. At this stage, mutant embryos showed various degrees of constriction at the extraembryonic-embryonic junction (Fig. 3). Mutant embryos that showed this constriction also manifested histological anomalies. In normal embryos, endoderm cells in the extraembryonic region are cuboidal and contain apical vacuoles, whereas those in the embryonic region are squamous. In the mutant embryos with the most marked constriction, however, endoderm cells in both the embryonic and extraembryonic regions are cuboidal and contain apical vacuoles (Fig. 3).
3K–M). Furthermore, whereas the mesoderm layer was present in the extraembryonic region (Fig. 3K), the normal organized structure of the ectoderm and mesoderm was not apparent in the embryonic region (Fig. 3L,M). Such histological anomalies were not detected in FoxH1−/− embryos that did not show a constriction (Fig. 3A–C). Mutant embryos in which the constriction was apparent but not pronounced showed relatively organized structures, although mesodermal cells accumulated near the junction between the embryonic and extraembryonic regions (Fig. 3F–H).

To determine the relation between the defects apparent at E7.5 and those observed at E8.5, embryos were recovered at E7.5 and allowed to develop in vitro for an additional 24 h. Embryos showing a severe constriction at E7.5 (Fig. 3N) developed the type III morphology after culture in vitro (Fig. 3O). Those showing a mild constriction (Fig. 3I) developed the type II morphology (Fig. 3J). Finally, FoxH1−/− embryos showing no constriction at E7.5 (Fig. 3D) developed the type I morphology (Fig. 3E). Therefore, embryos at stages earlier than E8.5 will hereafter also be referred to as type I, II, or III, accordingly.

Impaired orientation of the A-P axis in type III FoxH1−/− embryos

The constriction at the extraembryonic-embryonic junction of type III (and, to a lesser extent, of type II) FoxH1−/− embryos at E7.5 is reminiscent of the constriction that is a characteristic feature of HNF3β/FoxA2 (Ang and Rossant 1994; Weinstein et al. 1994), Lim1 (Shawlot and Behringer 1995), Otx2 (Acampora et al. 1995; Matsuo et al. 1995; Ang et al. 1998), and nodal (Varlet et al. 1997) mutants, all of which show defects in anterior specification caused by impaired function of the anterior visceral endoderm (AVE). We therefore examined type III (and type II) embryos for several AVE markers.

In normal embryos at E5.5, two AVE marker genes, Hex and lefty1, are initially expressed in the visceral endoderm at the distal tip (Thomas and Beddington 1996; Thomas et al. 1998; data not shown). The visceral endoderm cells expressing these genes subsequently migrate anteriorly to form the AVE by E6.5. Thus, Hex, Cer-l, Hesx1, and lefty1 are all expressed in the AVE at E6.75 (Fig. 4A,D,H,N). In contrast, Brachyury is initially expressed in the proximal epiblast, but its expression domain subsequently moves to the posterior side at E6.5 and marks the primitive streak (Fig. 4A). These complementary cell movements establish A-P polarity.

In type III FoxH1−/− embryos, however, Hex-expressing cells remained in the distal region at E6.75, with no evidence of movement toward the future anterior side (Fig. 4B). Cer-l (Fig. 4E), Hesx1 (Fig. 4I), and Lim-1 (data not shown) were also expressed in the visceral endoderm at the distal tip at this time, indicating that the AVE is incorrectly formed in the distal region. Conversely, the Brachyury expression domain failed to move to the posterior side, remaining in the proximal epiblast (Fig. 4B,E,I,O). Otx2 expression in the visceral endoderm was maintained in the mutant embryo (Fig. 4L). The expression of lefty1 in the visceral endoderm was abolished in the type III mutants (Fig. 4O), indicating that lefty1 expression in the endoderm may normally be induced by a Nodal-FoxH1 signaling pathway.

We also examined the type III embryo at E7.25. Whereas Brachyury expression remained in the proximal epiblast (Fig. 5C), expression of goosecoid, which marks the anterior primitive streak at this stage, was lost (Fig. 5I). FoxA2 expression, which also marks the anterior primitive streak, was absent, but its expression in the visceral endoderm was maintained (Fig. 5L,M). Furthermore, expression of lefty2, a marker for the nascent me-
soderm, was also absent [Fig. 5F], indicating the lack of the primitive streak. *Otx2*, which is initially expressed before gastrulation throughout the epiblast and becomes restricted to the anterior third of the embryo by E7.5 ([Ang et al. 1994]), was widely expressed in the ectoderm layer of type III embryos at E7.5 [data not shown]. These results indicate that proximal-distal (P-D) polarity is properly established in the type III mutants but that this polarity is not converted to the A-P axis. Incorrect orientation of the A-P axis is likely caused by impaired movement of the distal visceral endoderm. Consistent with this notion, sagittal sections of type III embryos (such as the one shown in Fig. 4E) revealed a marked accumulation of endoderm-like cells at the distal tip [data not shown]. These defects are highly similar to those of *Cripto* mutants ([Ding et al. 1998]), but with one important difference: Anterior neural fates (*Bf1* and *En1*-expressing cells) are induced in the distal region of *En1* mutants ([Ding et al. 1998]) but not in the corresponding region of type III *FoxH1*−/− embryos [data not shown]. The visceral endoderm cells at the distal end are enlarged in *Cripto* mutants ([Ding et al. 1998]), whereas massive endoderm cells accumulate in type III *FoxH1*−/− mutants [Fig. 4E].

Type II embryos show similar but less severe phenotypes at this stage of development. The expression of *Cer-1* in the visceral endoderm at E6.75 was down-regulated; the expression domain of this gene was apparent on the future anterior side but was located closer to the distal tip than in wild-type embryos [Fig. 4G]. These results indicate that A-P patterning is partially impaired and that the AVE is not fully functional in type II embryos, which likely explains why they lack anterior structures. In contrast, type I embryos showed normal expression patterns for *Brachyury* [Fig. 4C,F,J,P], *Hex* [Fig. 4C], *Cer-1* [Fig. 4F], *Hexx1* [Fig. 4I], and *Otx2* [Fig. 4M] but had lost *lefty1* expression in the AVE [Fig. 4P].

**Role of FoxH1 in Nodal signaling**

Histological examination indicated that type I *FoxH1*−/− embryos lack a definitive node, prechordal plate, and notochordal plate–notochord (Fig. 2H–J). To confirm these observations, we examined the expression at E8.5 of *Shh*, *Brachyury*, and *HNF3β* genes that are normally expressed in the node and its derivatives at this stage (Fig. 6A,C,E,I). In type I mutants, the expression of *Shh* [Fig. 6B,D] and *HNF3β* [Fig. 6I] was completely lost, and only sparse expression of *Brachyury* was apparent at the anterior midline [Fig. 6F], indicating a deficiency of node-derivied tissues. Type I embryos develop a single fused-head structure (pinhead) and specifically lack the most rostral portion, the forebrain. Thus, *Six3* expression, which is a marker for the forebrain [Fig. 6L], was abolished in type I mutants [Fig. 6M], whereas *Otx2* expression, which marks the forebrain and midbrain [Fig. 6N], was detected in these mutants [Fig. 6O]. In normal embryos, *Fgf8* is expressed in the forebrain, midbrain–hindbrain junction, and posterior streak at this stage ([Fig. 6C; Crossley and Martin 1995]). In type I embryos, however, *Fgf8* expression in the most anterior region was not apparent, although the other expression domains were preserved [Fig. 6H]. The lack of the forebrain is likely because of the absence of the prechordal plate, insufficient function of the AVE, or both. Truncation of anterior structures is more severe in type II embryos. Thus, *Otx2* expression [Fig. 6P] and *HNF3β* expression in the midline [Fig. 6K] are absent.

To investigate the mechanisms by which the absence of FoxH1 results in the failure of node formation, we examined the primitive streak of type I embryos at earlier stages. During normal gastrulation, *Brachyury* is expressed in the entire primitive streak, and its expression domain extends anteriorly with the extension of the primitive streak. In contrast, type II embryos show similar but less severe phenotypes. In type I embryos, however, *Shh* expression is limited to the anterior midline [Fig. 6F], indicating a deficiency of node-derived tissues. Type I embryos develop a single fused-head structure (pinhead) and specifically lack the most rostral portion, the forebrain. Thus, *Six3* expression, which is a marker for the forebrain [Fig. 6L], was abolished in type I mutants [Fig. 6M], whereas *Otx2* expression, which marks the forebrain and midbrain [Fig. 6N], was detected in these mutants [Fig. 6O]. In normal embryos, *Fgf8* is expressed in the forebrain, midbrain–hindbrain junction, and posterior streak at this stage ([Fig. 6C; Crossley and Martin 1995]). In type I embryos, however, *Fgf8* expression in the most anterior region was not apparent, although the other expression domains were preserved [Fig. 6H]. The lack of the forebrain is likely because of the absence of the prechordal plate, insufficient function of the AVE, or both. Truncation of anterior structures is more severe in type II embryos. Thus, *Otx2* expression [Fig. 6P] and *HNF3β* expression in the midline [Fig. 6K] are absent.

**Failure of primitive streak elongation and lack of the node in type I FoxH1−/− mutants**

Histological examination indicated that type I *FoxH1*−/− embryos lack a definitive node, prechordal plate, and notochordal plate–notochord (Fig. 2H–J). To confirm these observations, we examined the expression at E8.5 of *Shh*, *Brachyury*, and *HNF3β* genes that are normally expressed in the node and its derivatives at this stage (Fig. 6A,C,E,I). In type I mutants, the expression of *Shh* [Fig. 6B,D] and *HNF3β* [Fig. 6I] was completely lost, and only sparse expression of *Brachyury* was apparent at the anterior midline [Fig. 6F], indicating a deficiency of node-derived tissues. Type I embryos develop a single fused-head structure (pinhead) and specifically lack the most rostral portion, the forebrain. Thus, *Six3* expression, which is a marker for the forebrain [Fig. 6L], was abolished in type I mutants [Fig. 6M], whereas *Otx2* expression, which marks the forebrain and midbrain [Fig. 6N], was detected in these mutants [Fig. 6O]. In normal embryos, *Fgf8* is expressed in the forebrain, midbrain–hindbrain junction, and posterior streak at this stage ([Fig. 6C; Crossley and Martin 1995]). In type I embryos, however, *Fgf8* expression in the most anterior region was not apparent, although the other expression domains were preserved [Fig. 6H]. The lack of the forebrain is likely because of the absence of the prechordal plate, insufficient function of the AVE, or both. Truncation of anterior structures is more severe in type II embryos. Thus, *Otx2* expression [Fig. 6P] and *HNF3β* expression in the midline [Fig. 6K] are absent.

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streak (Fig. 5A; Kispert and Herrmann 1994). In type I mutants, the Brachyury expression domain was localized to the posterior side, but it failed to elongate anteriorly or distally (Fig. 5B). The expression of goosecoid and FoxA2, which mark the anterior primitive streak at this stage in normal embryos (Fig. 5G,J; Sasaki and Hogan 1993; Ang and Rossant 1994; Weinstein et al. 1994), was also examined. In the mutant embryos, goosecoid expression was greatly reduced and observed in the posterior-proximal region of the embryo proper (Fig. 5H). The expression of FoxA2 was also down-regulated and detected in the proximal region of the embryo proper (Fig. 5K), indicating that the anterior primitive streak was not properly specified. The expression of lefty2, which marks nascent mesoderm generated from the primitive streak (Fig. 5D; Meno et al. 1997), was also markedly down-regulated in type I mutants (Fig. 5E). Given that lefty2 expression is induced by a Nodal-FoxH1 signaling pathway, at least in the lateral plate at the early somite stage (Saijoh et al. 2000), this down-regulation of lefty2 may result directly from the loss of Nodal signaling. Together, these results indicate that formation of the primitive streak is initiated in type I embryos, but the anterior portion of the streak is not properly specified, resulting in node agenesis.

**Genetic interaction between FoxH1 and nodal**

The expression of nodal is induced by a Nodal-FoxH1 signaling pathway, at least in certain aspects of development, such as left-sided expression of this gene in the lateral plate at the early somite stage (Osada et al. 2000; Saijoh et al. 2000). Expression of nodal in the posterior-distal ectoderm during gastrulation also may be regulated by a FoxH1-dependent enhancer (Norris and Robertson 1999). We therefore examined nodal expression in FoxH1−/− embryos with the use of in situ hybridization. In wild-type embryos, this gene is initially expressed throughout the epiblast and in the underlying primitive endoderm at E5.5, but its expression in the epiblast becomes progressively restricted to the posterior region (Fig. 7A; Varlet et al. 1997). The expression of nodal disappears from the AVE at E6.75, but it is maintained in the lateral and posterior portions of visceral endoderm. It disappears from the posterior ectoderm and the visceral endoderm at E7.25 and then begins in the prospective dots.
Role of FoxH1 in Nodal signaling

Figure 7. Genetic interaction between FoxH1 and nodal. The expression of nodal in wild-type (A), type I FoxH1+/− (B), and type III FoxH1+/− (C) embryos was examined at E7.0 by whole-mount in situ hybridization. In type I embryos, nodal expression was greatly reduced and was confined to the proximal posterior ectoderm (B). In type III embryos (C), nodal expression was down-regulated and remained at the rim of the proximal epiblast. The frequencies of type III, type II, type I, and Smad2−/−-like phenotypes were also determined for FoxH1+/−, nodal+/− (upper bar) and FoxH1−/−, nodal+/− (lower bar) embryos at E8.5 [F]. The total numbers of the embryos examined are 152 and 28 for FoxH1+/−, nodal+/− and FoxH1−/−, nodal+/−, respectively. Most FoxH1−/−, nodal+/− embryos showed the type III morphology. Staining for β-galactosidase activity is also shown for FoxH1+/+, nodallacZ/+ [D] and FoxH1−/−, nodallacZ/+ [E] embryos at E7.0. Scale bars: 200 µm.

Role of FoxH1 in Nodal signaling

node. In type III FoxH1−/− embryos, however, nodal expression was down-regulated and remained at the rim of the proximal epiblast at E7.0, without being shifted to the posterior side [Fig. 7C]. In type I embryos, the abundance of nodal mRNA was also reduced [Fig. 7B]; the nodal expression site was shifted to the posterior side but was localized more proximally than in wild-type embryos. The down-regulation of nodal apparent in FoxH1−/− embryos indicates that the expression of this gene in the epiblast is maintained by a positive autoregulatory loop that includes FoxH1.

We also examined the potential genetic interaction between FoxH1 and nodal by crossing FoxH1 and nodallacZ mutants [Collignon et al. 1996]. Double heterozygotes [FoxH1+/−, nodal+/−lacZ/] appeared normal and were crossed with FoxH1+/− mice. The phenotype of the resulting FoxH1+/−, nodal+/−lacZ/+ embryos was more severe than that of FoxH1−/−, nodal+/−/lacZ/+ embryos [Fig. 7F]. Thus, most [24/28, 86%] of the FoxH1−/−, nodal+/−lacZ/+ embryos examined at E8.5 were type III, manifesting A-P patterning defects; the remaining embryos [4/28, 14%] resembled Smad2−/− mutant embryos, similar to the FoxH1−/− mutant shown in Figure 2O. As expected, nodal expression, which was monitored on the basis of the activity of the nodallacZ allele, remained in the proximal epiblast at E7.0 in all FoxH1+/−, nodallacZ/+ embryos examined [Fig. 7E].

Rescue of A-P patterning defects, but not midline defects, in FoxH1−/− embryos by expression of FoxH1 in extraembryonic tissues

We next examined whether FoxH1 is required in the epiblast lineage or in the extraembryonic lineage, including the primitive endoderm. The FoxH1 gene was specifically deleted from the epiblast with the use of the FoxH1floX allele and transgenic mice that express Cre in the epiblast and its derivatives but not in the primitive endoderm. The Cre-expressing transgenic mice harbor lefty2-Cre, a fusion construct comprising the Cre gene linked to the 5.5-kb upstream region of lefty2 [Fig. 8A]. When linked to the lacZ gene, the 5.5-kb upstream region of lefty2 confers expression in the nascent mesoderm at E6.5 to E7.0 and in the left lateral plate mesoderm at E8.25 [Sajioh et al. 1999]. However, one lefty2-Cre transgenic line [21B] showed epiblast-specific expression of Cre between E5.5 and E8.0 [lefty2 is not expressed in the epiblast]. Thus, crossing of line 21B animals with mice that harbor a Cre-sensitive lacZ reporter gene [Sakai and Miyazaki 1997] yielded embryos harboring both lefty2-Cre and the lacZ reporter gene that showed β-galactosidase activity throughout the epiblast lineage but not in the extraembryonic tissues, including the primitive endoderm, both at E6.5 [Fig. 8B,C] and E7.0 [Fig. 8D,E].

We crossed FoxH1+/− animals harboring the Cre transgene with FoxH1floX/floX mice and genotyped the resulting embryos by PCR analysis of yolk sac DNA. FoxH1+/−, lefty2-Cre embryos were first examined at E8.5; most [15/16, 94%] of these embryos showed the type I phenotype, having a single fused head [Fig. 8J–L]. We also examined FoxH1+/−, lefty2-Cre embryos at an earlier stage (E7.0). Again, they [3/3] showed the type I morphology, showing normal Cer-1 expression in the AVE region [Fig. 8N]; type III embryos showing Cer-1 expression at the distal end [such as the one shown in Fig. 4B] were not detected [0/3] at this stage. Unexpectedly, lefty1 expression in the visceral endoderm was lost in the epiblast-specific FoxH1 mutant embryos [Fig. 8P], indicating that the expression of lefty1 is induced by...
FoxH1-dependent signals (most likely, Nodal signals) derived from the epiblast. Type II embryos were not detected at either E8.5 or E7.0. These results indicate that the presence of FoxH1 in the primitive endoderm is able to rescue the A-P patterning defects of type III and type II embryos but not the anterior primitive streak defects of type I embryos.

We also examined chimeric embryos that were generated by injecting FoxH1+/− ES (ROSA26) cells into FoxH1−/− blastocysts [Fig. 9]. Among such chimeras with extensive colonization of ROSA26 ES cells, about a half of them [5/9] showed severe A-P patterning defects characteristic of type III phenotype [Fig. 9J,K]. In the remaining chimeric embryos, midline defects were mostly rescued [Fig. 9E-I]. Thus, the node and notochordal plate were formed [data not shown], and somites on the both sides were separated by the midline [Fig. 9I]. The neural plate was folded along the A-P axis [Fig. 9G], but the most rostral part of the neural plate remained unfolded [Fig. 9H]. Therefore, injection of wild-type ES cells could rescue midline defects but failed to rescue A-P patterning defects.

These results indicate that FoxH1 in the primitive endoderm is required for A-P patterning, whereas FoxH1 in the epiblast is essential for primitive streak formation. The expression pattern of FoxH1 is consistent with this notion; FoxH1 was expressed both in the visceral endoderm and in the epiblast, but not in the extraembryonic region, of wild-type embryos at E6.5 to E7.0 [Figs. 8F–I]. Therefore, among the nonepiblast tissues, it is most likely in the visceral endoderm that FoxH1 plays a role in A-P patterning.

**Discussion**

Our analysis of FoxH1 mutant mice indicates that FoxH1 is the major transcriptional transducer of Nodal signaling in early development. This transcription factor...
appears to play multiple roles: Its activity in the primitive endoderm and in the epiblast is essential for A-P patterning and for node formation, respectively. A zebrafish mutant (schumalspur, or sur) that is deficient in FoxH1 has been described recently (Pogoda et al. 2000; Sirotkin et al. 2000). This mutant lacks an organizer and shows defects in dorsal axial structures that are equivalent to the defects observed in type I FoxH1−/− mice.

FoxH1-dependent signals in visceral endoderm are required for orienting the A-P axis

The A-P axis is established by three sequential steps: [1] graded expression of several genes along the P-D axis of the embryo, [2] movement of the distal visceral endoderm toward the anterior end of the embryo, and [3] specification of the underlying epiblast to an anterior identity by AVE-derived signals (Beddington and Robertson 1998, 1999; Kimura et al. 2000).

In type III FoxH1−/− embryos, the P-D axis is established properly in the egg cylinder structure, but the distal visceral endoderm fails to migrate anteriorly. Epiblast-specific deletion of FoxH1 indicated that FoxH1 in the visceral endoderm is essential for cell movement. The FoxH1-dependent signals may be provided by Nodal, a notion that is supported by previous observations by other researchers. Thus, type III FoxH1 mutants show A-P patterning defects similar to those of mouse mutants that lack Cripto (Ding et al. 1998), which functions as a coreceptor for Nodal (Sakuma et al., in prep.). Furthermore, chimeric embryos consisting of wild-type epiblast and ALK4−/− endoderm (Gu et al. 1998) fail to undergo gastrulation and show defects similar to those of type III FoxH1 mutants (ALK4 is most likely a type I receptor for Nodal; R. Sakuma, Y. Ohnishi, C. Meno, and H. Hamada, in prep.). The mechanism by which FoxH1-dependent signals [Nodal signals] promote the anterior-directed migration of the distal visceral endoderm remains unknown. One possibility is that Nodal activity may be distributed unevenly along the future A-P axis in the region of the distal visceral endoderm, and this differential Nodal activity may generate differences in cell proliferation or in the orientation of cell division.

Formation of the anterior neural structures requires one additional step: stabilization of anterior identity by signals derived from the prechordal plate (Rhinn et al. 1998).
1998, Shawlot et al. 1999, Tam and Steiner 1999). The absence of the forebrain in type I FoxH1−/− embryos may result from the lack of the prechordal plate, which comprises axial mesoderm cell populations derived from the anterior streak. Consistent with this conclusion, epiblast-specific deletion of FoxH1 was sufficient to give rise to type I embryos lacking the forebrain. However, impairment of AVE function can also induce a similar phenotype, as evident in chimeric nodal−/− embryos that show extensive colonization of wild-type ES cells (Varlet et al. 1997). Therefore, the absence of the forebrain in type I embryos might also result from dysfunction of the AVE. Indeed, the forebrain was also impaired in chimeric type I embryos that are derived from the anterior streak. Consistent with this conclusion, expression factors other than FoxH1 during streak migration may be delayed. Thus, their mutants do not show A-P patterning defects at later stages. The most likely reason for this difference is genetic background. Our mutant mice have a 129/B6 mixed background, whereas their mutant mice have a 129/CD-1 mixed background. Genetic interaction between FoxH1 and nodal indicates that both FoxH1-dependent and independent pathways mediate Nodal signaling during A-P patterning. Perhaps, FAST-independent pathway can complement the absence of FoxH1 in some genetic backgrounds but not in others.

Role of FoxH1 in formation and patterning of the primitive streak

Despite extensive studies in various vertebrates, little is known of the mechanism by which formation of the primitive streak is initiated in the mouse. The observations that mice with mutations in nodal or in genes for its putative receptors, such as ActRIIA, ActRIIB, and ActRIIA, fail to gastrulate (Conlon 1994; Gu et al. 1998; Song et al. 1999) indicate that Nodal signals are essential for this process. However, type I and type II FoxH1−/− embryos formed the primitive streak, and posterior development was relatively normal in these animals, suggesting that FoxH1 is dispensable for streak formation. Nodal signaling is thus likely mediated by transcription factors other than FoxH1 during streak formation.

The primitive streak is initially formed in a small region near the extraembryonic-embryonic junction and elongates distally during gastrulation. The streak is patterned along the A-P axis, and cells derived from this structure are allocated to various mesodermal lineages, depending on their stage and position. For instance, cells derived from the early streak contribute predominantly to extraembryonic mesoderm, and the anterior portion of the mid-to-late primitive streak contributes to the node (Lawson et al. 1986; Tam and Beddington 1987). However, little is known of the mechanisms that underlie these patterning events.

The primitive streak of type I FoxH1−/− mutants failed to elongate and lacked the anterior portion. The truncation of the streak in these embryos is likely because of down-regulation of nodal expression in the posterior ectoderm. Thus, nodal expression was abolished in the distal-posterior epiblast and was markedly reduced in the proximal-posterior region of the epiblast in the type I mutants. These observations on nodal expression are consistent with the results of recent studies on the transcriptional regulation of nodal (Adachi et al. 1999; Norris and Robertson 1999). The expression of nodal in the ectoderm is controlled by at least two enhancers: The FoxH1-dependent enhancer (referred to as ASE) induces expression in the posterior ectoderm, whereas the other enhancer induces expression in the proximal epiblast. The lack of FoxH1 would therefore be expected to reduce nodal expression in the posterior ectoderm. In conclusion, FoxH1 is not essential for the initiation of primitive streak formation. However, it plays an important role in elongation and patterning of the streak; specifically, it maintains nodal expression in the anterior portion of the streak by acting as a component of a Nodal autoregulatory loop.

In other vertebrates, such as Xenopus and the chicken, the organizer is induced by synergistic stimulation by Wnt and Nodal-Activin–like signals (Harland and Gerhart 1997). In frog and zebrafish, a Nodal-FoxH1 pathway induces the expression of organizer-associated genes (Toyama et al. 1995; Watanabe and Whitman 1999). Furthermore, FoxH1 mutants (sur) in zebrafish fail to form a gastrula organizer (Pogoda et al. 2000; Sirokin et al. 2000). These observations and our analysis of FoxH1 mutant mice indicate that a Nodal-FoxH1 signaling pathway plays a conserved role in organizer formation in vertebrates.

FoxH1 mediates Nodal signaling during early mouse development

FoxH1 was initially identified as a mediator of Activin signaling (Chen et al. 1996). In cultured cells or frog animal caps, FoxH1 interacts with Smad2 (or Smad3) and Smad4 and mediates signaling by TGF-β and TGF-β-related factors such as Activin and Nodal (Chen et al. 1996; Labbe et al. 1998; Weisberg et al. 1998; Zhou et al. 1998; Liu et al. 1999; Saijoh et al. 2000). However, our data now indicate that FoxH1 plays the major role in mediating Nodal signaling during early development of the mouse. FoxH1−/− mice showed various patterning defects that have been observed previously in mutant mice lacking other components of the Nodal signaling pathway. Thus, type III FoxH1−/− embryos manifested A-P patterning defects similar to those apparent in Cripto mutants (Ding et al. 1998). In addition, similar to Cripto−/− mice (Ding et al. 1998) and Smad2−/− chimeric mice (Tremblay et al. 2000), type II and type I FoxH1−/− embryos lacked definitive endoderm. Furthermore, the phenotype of type I FoxH1−/− embryos was similar to...
that of chimeric nodal\(^{-/-}\) embryos with a small contribution of wild-type ES cells [Varlet et al. 1997]. Genetic interaction was apparent between FoxH1 and nodal. Consistent with the suggestion that expression of nodal and lefty2 is induced by a Nodal-FoxH1 pathway [Sajioh et al. 2000], we showed that nodal expression was initiated but down-regulated in type III and type I FoxH1\(^{-/-}\) embryos and that lefty2 expression was markedly reduced in the type III and type I mutants. The expression of lefty1 in the visceral endoderm also may be induced by a Nodal-FoxH1 pathway, given that expression of this gene in this region was lost in type III FoxH1\(^{-/-}\) embryos and epiblast-specific FoxH1 mutant embryos.

Despite the similarities between the phenotypes of FoxH1\(^{-/-}\) mutants and mutants lacking other components of the Nodal signaling pathway, substantial differences are also apparent. In Cripto mutants, the AVE incorrectly formed at the distal tip acts on the underlying epiblast and induces anterior neural fates in the absence of streak-derived tissues [Ding et al. 1998]. In contrast, such anterior neural identity was not induced in type III FoxH1\(^{-/-}\) mutants. This apparent discrepancy is not easily reconciled, but one possible explanation is that Cripto plays a role in addition to functioning as a co-receptor for Nodal signaling. For instance, Cripto may confer lability on signals from the visceral endoderm, as suggested previously by others [Shawlot et al. 1999]. The patterning defects of FoxH1\(^{-/-}\) mutants are less severe than those of embryos completely lacking Nodal. Thus, the extraembryonic tissues are highly abnormal in nodal\(^{-/-}\) mutants, whereas those tissues are relatively normal in type III FoxH1 mutants. The phenotype of FoxH1\(^{-/-}\) mutants is also less severe than that of Smad2 mutants. In the absence of Smad2, embryos fail to establish P-D polarity properly, and the entire epiblast adopts an extraembryonic mesodermal fate [Waldrip et al. 1998; Heyer et al. 1999]. In contrast, FoxH1\(^{-/-}\) embryos establish P-D polarity. These phenotypic differences among nodal, Smad2, and FoxH1 mutants suggest that Nodal signals act through both FoxH1-dependent and FoxH1-independent pathways. The actions of Nodal in A-P patterning and in patterning and elongation of the primitive streak are FoxH1 dependent, whereas those in mesoderm induction are FoxH1 independent.

Nodal signaling is also implicated in left-right patterning at a later stage of development [Schier and Shen 2000], and FoxH1 may play a role in mediating Nodal signals during this process. However, the early death of FoxH1 mutant mice prevented us from studying the role of this transcription factor in left-right asymmetry. FoxH1 also may mediate signaling by TGF-\(\beta\) and related factors at later stages of development. Clarification of the roles of FoxH1 at these later stages will require conditional deletion of the gene.

Materials and methods

Generation of FoxH1-deficient mice

Genomic FoxH1 clones were isolated from a genomic DNA library constructed from E14 ES cells. A targeting vector was constructed by subcloning the 5’ flanking region [the 3.9-kb Sall–SacII fragment], the exon 1–intron 1 region [the 2.3-kb SacII–Smad fragment], and the region containing the other exons and intron as well as the 3′ flanking region [the 7.8-kb Smad–XbaI fragment] of FoxH1 into a modified pMC1-DTpa vector (Taniguchi et al. 1997). A loxP fragment containing a BamHI site was inserted between exon 1 and exon 2, and a loxP-Frt-neo-Frt cassette [Meyers et al. 1998] was inserted into the SacII site in the 5′ flanking region. Gene targeting was performed as described [Sawai et al. 1991]. The targeting vector was linearized with NotI before introduction into R1 ES cells by electroporation.

Of 140 G418-resistant ES clones, two clones (F94, F128) were shown to have undergone homologous recombination, as confirmed by Southern blot analysis with various probes, including a 5′ probe, a 3′ probe, and a neo probe. To generate a flox allele, we subjected F128 cells to electroporation with a Flp expression vector [pCAGGS-Flpe-ires-puro, kindly provided by F. Stewart and S. Dynecki] followed by selection with puromycin [1 \(\mu\)g/mL Sigma]. Flp-mediated deletion was verified by PCR and Southern blot analyses. PCR was performed with the primers P1 (5′-ATCCTCGCCATGGCAACGCGA) and P2 (5′-AGTACCA CAGAATAGCAGCAG), wild-type and flox alleles yield fragments of 252 and 361 bp, respectively. One clone (F128-10) that was shown to have lost neo was used in the present study. F94 or F128-10 cells were injected into blastocysts of C57BL/6Cr mice [BOIN, Shizuoka Japan], resulting in the birth of chimeric animals. Male chimeras derived from each ES cell line were bred with C57BL/6Cr females, yielding heterozygous F1 offspring (C57BL/6Cr × 129 background). To generate a null allele [FoxH1\(^{-/-}\)], we crossed male chimeras with CAG-Cre transgenic mice [Sakai and Miyazaki 1997] to excise the loxP cassette. The resulting F1, offspring were verified by Southern hybridization and PCR analysis with primers P1, P2, and P3 (5′-GACTGCGTGGCGTGAAGGGC-3′), wild-type and excised alleles yield fragments of 252 and 592 bp, respectively. The F1 heterozygotes were crossed with each other, producing FoxH1\(^{neo/+}\), FoxH1\(^{flox/+}\), and FoxH1\(^{-/-}\) mice. For RT-PCR analysis, total RNA was prepared from E7.75 embryos with guanidine isocyanate, and was reverse transcribed with oligo [dT]. For detecting FoxH1 mRNA, cDNA was subjected to PCR with the primers 5′-ATCCGTCAGCGTCACGACATG-3′ and 5′-CTTGCGCAGAAGGCTCTGTG-3′. To detect Hprt mRNA as a control, the same cDNA was amplified with the primers 5′-AGCCATGTAGAACCAGGTTA-3′ and 5′-GTTGAGAGAT CATCTCCACC-3′.

In situ hybridization and histology

Mouse embryos were staged on the basis of their morphology [Downs and Davies 1993]. Whole-mount in situ hybridization was performed according to standard procedures [Wilkinson 1992]. Wild-type and mutant embryos were processed in the same tube. Embryos were genotyped by PCR analysis of yolk sac DNA with primers P1 and P2 for the wild-type FoxH1 allele, and with primers P1 and P3 for the FoxH1 null allele. For histology, embryos were fixed with Bouin’s solution, embedded in paraffin, and sectioned at a thickness of 8 \(\mu\)m. Sections were stained with hematoxylin and eosin.

Analysis of genetic interaction between FoxH1 and nodal

Mice that contain an IRES-lacZ cassette in the second exon of nodal have been described previously [Collignon et al. 1996]. We crossed nodal\(^{-/-}\) mice with FoxH1\(^{-/-}\) mice to obtain...
double heterozygotes. Embryos obtained by intercrossing of the double heterozygotes or by crossing the double heterozygotes with FoxH1<sup>−/−</sup> animals were analyzed. The genotype of each embryo was determined by PCR. The expression of nodal in these embryos was monitored by staining with X-Gal.

Whole-embryo culture

E7.5 embryos were cultured for 24 h in 50-mL disposable tubes containing 2 mL of 50% Dulbecco's modified Eagle's medium supplemented with 5% fetal serum, as described [Lawson et al. 1986]; this volume of medium was sufficient for culturing four embryos [Sturm and Tam 1993]. The tubes were filled with a mixture of 5% CO<sub>2</sub>, 5% O<sub>2</sub>, and 90% N<sub>2</sub> and were rotated at 30 rpm on a roller apparatus in an incubator.

Epiblast-specific deletion of FoxH1

The <i>lefty2-Cre</i> transgene was constructed by ligating the 5.5-kb upstream region of <i>lefty2</i> to a Cre cassette derived from pBS-Cre [kindly provided by H. Kondoh]. Several mouse lines containing this transgene were established. To examine the specificity of Cre expression, we crossed each line with transgenic mice that harbor a lacZ gene that can be expressed only after Cre-mediated excision. Embryos were genotyped and stained with X-Gal. One transgenic line (21B) that shows epiblast-specific expres-

Generation and analysis of chimera embryos

Chimeras were generated by blastocyst injection as described [Bradley 1987]. Blastocysts were collected from intercross between FoxH1<sup>+/−</sup> animals and were injected with wild-type ES [ROSA26] cells at a ratio of 2:14 ES cells/blastocyst. Chimeric embryos were recovered at E8.5, fixed and processed for l<sup>acZ</lsup> expression, we crossed each line with transgenic mice that show epiblast-specific expres-

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References

Acampora, D., Mazan, S., Lallemand, Y., Avantaggiato, V., Maury, M., Simeone, A., and Brulet, P. 1995. Forebrain and midbrain regions are deleted in <i>Otx2</i><sup>−/−</sup> mutants due to a defective anterior neuroectoderm specification during gastrulation. Development 121:3279–3290.

Adachi, H., Saijoh, Y., Mochida, K., Ohishi, S., Hashiguchi, H., Hirao, A., and Hamada, H. 1999. Determination of left-right asymmetric expression of nodal by a left side-specific enhancer with sequence similarity to a <i>lefty</i>-2 enhancer. Genes & Dev. 13:1589–1600.

Ang, S.-L. and Rossant, J. 1994. HNF3β is essential for node and notochord formation in mouse development. Cell 78:561–574.

Ang, S.-L., Conlon, R.A., Jin, O., and Rossant, J. 1994. Positive and negative signals from mesoderm regulate the expression of mouse Otx2 in ectoderm explants. Development 120:2979–2989.

Ang, S.-L., Jin, O., Rhinn, M., Daigle, N., Stevenson, L., and Rossant, J. 1998. A targeted mouse Otx2 mutation leads to severe defects in gastrulation and formation of axial mesoderm and to deletion of rostral brain. Development 122:243–252.

Beddington, R. and Robertson, E.J. 1998. Anterior patterning. Trends Genet. 14:277–284.

Bradley, A. 1987. Production and analysis of chimeric mice. In Teratocarcinomas and embryonic stem cells: A practical approach (ed. E.J. Robertson), pp. 131–151. IRL Press, Oxford, UK.

Chen, X., Rubock, M.J., and Whitman, M. 1996. A transcriptional partner for Mad proteins in TGFβ signaling. Nature 383:691–696.

Chen, X., Weisberg, E., Fridmacher, V., Watanabe, M., Naco, G., and Whitman, M. 1997. Smad4 and FAST-1 in the assembly of activin-response factor. Nature 389:85–89.

Collignon, J., Varlet, I., and Robertson, E.J. 1996. Relationship between asymmetric nodal expression and the direction of embryonic turning. Nature 381:155–158.

Conlon, F.L., Lyons, K.M., Takaesu, N., Barth, K.S., Kispert, A.K., Herrmann, B., and Robertson, E.J. 1994. A primary requirement of nodal in the formation and maintenance of the primitive streak in mouse. Development 120:1919–1928.

Crossley, P.H. and Martin, G.R. 1995. The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. Development 121:439–451.

Ding, J., Yang, L., Yan, Y.-T., Chen, A., Desai, N., Wynshaw-Boris, A., and Shen, M.M. 1998. <i>Crypto</i> is required for correct orientation of the anterior-posterior axis in the mouse embryo. Nature 395:702–707.
Downs, K.M. and Davies, T. 1993. Staging of gastrulating mouse embryos by morphological landmarks in the dissecting microscope. Development 118: 1255–1266.

Gritsman, K., Zhang, J., Cheng, S., Heckscher, E., Talbot, W.S., and Schier, A.F. 1999. The EGF-CFC protein one-eyed pinhead is essential for Nodal signaling. Cell 97: 121–132.

Gu, Z., Nomura, M., Simpson, B.B., Lei, H., Feijen, A., Van den Eijnden-van Raaij, J., Donohoe, P.K., and Li, E. 1998. The type III activin receptor ActRIIB is required for egg cylinder organization and gastrulation in the mouse. Genes & Dev. 12: 844–857.

Harland, R. and Gerhart, J. 1997. Formation and function of Spemann’s organizer. Annu. Rev. Cell. Dev. Biol. 13: 611–667.

Heyer, J., Escalante-Alcade, D., Lia, M., Boettginger, E., Edelman, W., Stewart, C., and Kucherlapati, R. 1999. Postgastrulation Smad2-deficient embryos show defects in embryo turning and anterior morphogenesis. Proc. Natl. Acad. Sci. 96: 12595–12600.

Hogan, B., Beddington, R., Costantini, F., and Lacy, E. 1994. Manipulating the mouse embryo: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY.

Hoodless, P., Pye, M., Chaxaud, C., Labbe, E., Attisano, L., Rossant, J., and Wrana, J.L. 2001. Fast functions to specify the anterior primitive streak in the mouse. Genes & Dev. 15: 1257–1271.

Kimura, C., Yoshinaga, K., Tian, E., Suzuki, M., Aizawa, S., and Matsuo, I. 2000. Visceral endoderm mediates forebrain development by suppressing posteriorizing signals. Dev. Biol. 225: 304–321.

Kispert, A. and Herrmann, B.G. 1994. Immunohistochemical analysis of the Brachyury protein in wild-type Ildl mutant mouse embryos. Dev. Biol. 161: 179–193.

Labbé, E., Silvestri, C., Hoodless, P.A., Wrana, J.L., and Attisano, L. 1998. Smad2 and Smad3 positively and negatively regulate TGFβ-dependent transcription through the fork-head DNA-binding protein FAST2. Mol. Cell. 2: 109–120.

Lawson, K.A., Meneses, J.J., and Pedersen, R.A. 1986. Cell fate regulation in the endoderm of the presomite mouse embryo, studied with an intercellular tracer. Dev. Biol. 115: 325–339.

Liu, B., Dou, C.L., Prabhu, L., and Lai, E. 1999. FAST2 is a mammalian winged-helix protein which mediates TGFβ signal transduction. Mol. Cell. 19: 424–430.

Massague, J. 1998. TGFβ signal transduction. Annu. Rev. Biochem. 67: 753–791.

Matsuo, I., Kuratani, S., Kimura, C., Takeda, N., and Aizawa, S. 1995. Mouse Otx2 functions in the formation and patterning of rostral head. Genes & Dev. 9: 2646–2658.

Meno, C., Ito, Y., Saijoh, Y., Matsuda, Y., Tashiro, K., and Hamada, H. 1997. 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 2: 513–524.

Meyers, E.N., Lewandoski, M., and Martin, G.R. 1998. An Fgfl mutant allelic series generated by Cre- and Flp-mediated recombination. Nature Genet. 18: 136–141.

Nomura, M. and Li, E. 1998. Smad2 role in mesoderm formation, left-right patterning and craniofacial development. Nature 393: 786–790.

Norris, D.P. and Robertson, E.J. 1999. Node-specific and asymmetric nodal expression patterns are controlled by two distinct cis-acting regulatory elements. Genes & Dev. 13: 1575–1589.

Osada, S., Saijoh, Y., Frisch, A., Yeo, C.-Y., Adachi, H., Watanabe, M., Whitman, M., Hamada, H., and Wright, C.V. 2000. Activin/Nodal responsiveness and asymmetric expression of Xenopus nodal-related gene converge on a FAST-regulated module in intron 1. Development 127: 2503–2514.

Piccolo, S., Agius, E., Ledys, N., Bhattacharyya, S., Grunz, H., Boumeester, T., and De Robertis, E.M. 1999. The head inducer Cerberus is a multifunctional antagonist of Nodal, BMP, and Wnt signals. Nature 397: 707–710.

Pogoda, H.-M., Solnica-Krezel, L., Driever, W., and Meyer, D. 2000. The zebrafish forkhead transcription factor FoxH1/ Fast1 is a modulator of Nodal signaling required for organizer formation. Curr. Biol. 10: 1041–1049.

Rhim, M., Dierich, A., Shawlot, W., Behringer, R.R., Le Meur, M., and Ang, S.L. 1998. Sequential roles for Otx2 in visceral endoderm and neuroectoderm for forebrain and midbrain induction and specification. Development 125: 845–856.

Saijoh, Y., Adachi, H., Mochida, K., Ohishi, S., Hirao, A., and Hamada, H. 1999. Distinct transcriptional regulatory mechanisms underlie left-right asymmetric expression of lefty-1 and lefty-2. Genes & Dev. 13: 259–269.

Sakai, K. and Miyazaki, J. 1997. A transgenic mouse line that retains Cre recombinase activity in mature oocytes irrespective of the Cre transgene transmission. Biochem. Biophys. Res. Commun. 237: 318–324.

Sasaki, H. and Hogan, B.L.M. 1993. Differential expression of multiple fork head related genes during gastrulation and axial pattern formation in the mouse embryo. Development 118: 47–59.

Sawai, S., Shimono, A., Hanaoka, K., and Kondo, H. 1991. Embryonic lethality resulting from disruption of both N-myc alleles in mouse zygotes. New Biologist 9: 861–869.

Schier, A.F. and Shen, M.M. 2000. Nodal signaling in vertebrate development. Nature 403: 385–389.

Shawlot, W. and Behringer, R.R. 1995. Requirement for Lim1 in head organizer function. Nature 374: 425–430.

Shawlot, W., Wakamiya, M., Kwan, K.M., Kania, A., Jessell, T., and Behringer, R.R. 1999. Lim1 is required in both primitive streak-derived tissues and visceral endoderm for head formation in the mouse. Development 126: 4925–4932.

Sirotkin, H.L., Gates, M.A., Kelley, P.D., Schier, A.F., and Talbot, W.S. 2000. Fast1 is required for the development of dorso- lateral axial structures in zebrafish. Curr. Biol. 10: 1051–1054.

Song, J., Oh, S.P., Schrewe, H., Nomura, M., Lei, H., Okano, H., Gridley, T., and Li, E. 1999. The type II activin receptors are essential for egg cylinder growth, gastrulation, and rostral head development in mice. Dev. Biol. 213: 157–169.

Sturm, K. and Tam, P.P.L. 1993. Isolation and culture of whole postimplantation embryos and germ layer derivatives. In Guide to techniques in mouse development (eds. P.M. Wassarman and M.L. DePamphilis), pp. 164–190. Academic Press, San Diego, CA.

Tam, P.P.L. and Beddington, R.S.P. 1987. The formation of mesodermal tissues in the mouse embryo during gastrulation and early organogenesis. Development 99: 109–126.

Tam, P.P.L. and Steiner, K.A. 1999. Anterior patterning by synergistic interaction of the early gastrula organizer and the anterior germ layer tissues of the mouse embryo. Developmen-
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Taniguchi, M., Yuasa, S., Fujisawa, H., Naruse, I., Saga, S., Mishima, M., and Yagi, T. 1997. Disruption of Semaphorin III/D gene causes severe abnormality in peripheral nerve projection. Neuron 19: 519–530.

Thomas, P. and Beddington, R. 1996 Anterior primitive endoderm may be responsible for patterning the anterior neural plate in the mouse embryo. Curr. Biol. 6: 1487–1496.

Thomas, P.Q., Brown, A., and Beddington, R.S. 1998. Hex: A homeobox gene revealing peri-implantation asymmetry in the mouse embryo and an early transient marker of endothelial cell precursors. Development 125: 85–94.

Toyama, R., O’Connell, M.L., Wright, C.V., Kuehn, M.R., and Dawid, I.B. 1995. Nodal induces ectopic goosecoid and lim1 expression and axis duplication in zebrafish. Development 121: 383–391.

Tremblay, K.D., Hoodless, P.A., Bikoff, E.K., and Robertson, E.J. 2000. Formation of the definitive endoderm in mouse is a Smad2-dependent process. Development 127: 3079–3090.

Varlet, I., Collignon, J., and Robertson, E.J. 1997. nodal expression in the primitive endoderm is required for the specification of the anterior axis during mouse gastrulation. Development 124: 1033–1044.

Waldrip, W.R., Bikoff, E.K., Hoodless, P.A., Wrana, J.L., and Robertson, E.J. 1998. Smad2 signaling in extraembryonic tissues determines anterior-posterior polarity of the early mouse embryo. Cell 92: 797–808.

Watanabe, M. and Whitman, M. 1999. FAST-1 is a key maternal effector of mesoderm inducers in the early Xenopus embryo. Development 126: 5621–5634.

Weinstein, D.C., Ruiz i Altaba, A., Chen, W.S., Hoodless, P., Prezioso, V.R., Jessel, T.M., and Darnell, J.E., Jr. 1994. The winged-helix transcription factor HNF3β is required for notochord development in the mouse embryo. Cell 78: 575–588.

Weisberg, E., Winner, G.E., Chen, X., Farnsworth, C., Hogan, B.L.M., and Whitman, M. 1998. A mouse homologue of FAST-1 transduces TGFβ superfamily signals and is expressed during early embryogenesis. Mech. Dev. 79: 17–27.

Whitman, M. 1998. Smads and early developmental signaling by the TGFβ superfamily. Genes & Dev. 12: 2445–2462.

Wilkinson, D.G. 1992. Whole mount in situ hybridization of vertebrate embryos. In In situ hybridization: a practical approach (ed. D.G. Wilkinson), pp. 75–84. IRL Press, Oxford, UK.

Zhou, S., Zawel, L., Lengauer, C., Kinzler, K.W., and Vogelstein, B. 1998. Characterization of human FAST-1, a TGFβ and activin signal transducer. Mol. Cell 2: 121–127.

Zhou, X., Sasaki, H., Lowe, L., Hogan, B.L., and Kuehn, M.R. 1993. Nodal is a novel TGF-β-like gene expressed in the mouse node during gastrulation. Nature 361: 543–547.
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