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Nodal signaling: developmental roles and regulation

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Nodal-related ligands of the transforming growth factor-beta (TGFβ) superfamily play central roles in patterning the early embryo during the induction of mesoderm and endoderm and the specification of left-right asymmetry. Additional roles for this pathway in the maintenance of embryonic stem cell pluripotency and in carcinogenesis have been uncovered more recently. Consistent with its crucial developmental functions, Nodal signaling is tightly regulated by diverse mechanisms including the control of ligand processing, utilization of co-receptors, expression of soluble antagonists, as well as positive- and negative-feedback activities.

Introduction

The Nodal signaling pathway is integral to processes of pattern formation and differentiation that take place during the pre-gastrulation and gastrulation stages of chordate development. In particular, Nodal signaling is essential for the specification of the primary body axes, as well as for the formation of mesoderm and endoderm. Its central importance has been established using molecular genetic studies in the frog, zebrafish, chick and mouse systems, and its functional conservation has been demonstrated in various species. Here, I describe the central components and molecular properties of the Nodal pathway and delineate general mechanisms of its function during embryogenesis, with a focus on recent findings.

Key components of the Nodal signaling pathway

Nodal pathway ligands are members of the transforming growth factor-beta (TGFβ) superfamily that bind to type I and type II serine-threonine kinase receptors, and signal through the Smad2/Smad3 branch of the TGFβ pathway (Schier, 2003; Schier and Shen, 2000; Whitman, 2001) (Table 1). Activated type I receptors phosphorylate cytoplasmic Smad2 and/or Smad3, leading to their interaction with Smad4 and the subsequent formation of transcriptional complexes in the nucleus. Unique to the Nodal pathway are co-receptors of the EGF-CFC family, which are small cysteine-rich extracellular proteins that are attached to the plasma membrane through a glycosyl-phosphatidylinositol (GPI) linkage and are essential for Nodal signaling (Shen and Schier, 2000). Furthermore, Nodal signaling can be antagonized by soluble inhibitors of the Lefty subclass of TGFβ factors (Table 1), and is mediated by FoxH1 and Mixer transcriptional activators (Fig. 1). Importantly, Nodal and the TGFβ ligand Activin often elicit similar responses in gain-of-function studies, but differ in that Nodal signaling is EGF-CFC-dependent and can be blocked by Lefty inhibitors. (In cases in which Activin and Nodal are likely to have indistinguishable effects, I will refer to 'Activin/Nodal' pathway activity.)

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Nodal and related ligands

Nodal ligands were originally identified through forward genetic screens in mouse and zebrafish (Box 1). Whereas a single Nodal ligand is found in mouse, human and chick, there are multiple Nodal-related ligands in frogs (encoded by six *xNr* genes) and zebrafish [*cyclops* (*cyc*; *ndr2* – Zebrafish Information Network), *squint* (*sqt*; *ndr1* – Zebrafish Information Network) and *southpaw* (*spw*)] (Schier, 2003). Except for *xNr3*, which may function independently of the Nodal signaling pathway, the Nodal-related genes in fish and frogs appear to perform the same core functions as Nodal ligands in amniotes.

Interestingly, recent studies have shown that additional TGFβ ligands can utilize the core components of this pathway and generate Nodal-like responses in vivo (Chen et al., 2006; Cheng et al., 2003). One such ligand is *Xenopus* Vg1, whose role in mesoderm and endoderm formation has only recently been clarified (Birsoy et al., 2006), and whose conserved functions appear to have been split between its mammalian counterparts growth differentiation factor 1 (Gdf1) and Gdf3 (Andersson et al., 2006; Chen et al., 2006). Null mutants for *Gdf3* have phenotypes resembling those of mutants of Nodal pathway components (Chen et al., 2006), and double-mutant analyses have revealed partially overlapping functions of *Gdf1* and *Nodal* (Andersson et al., 2006). Notably, signaling by zebrafish Vg1 (Vtg1) and mouse Gdf1 in microinjected zebrafish embryos and by Gdf3 in cell culture is EGF-CFC-dependent (Chen et al., 2006; Cheng et al., 2003); however, Gdf3 function may be complex, as it can also antagonize BMP signaling (Levine and Brivanlou, 2006). By contrast, the *Xenopus* TGFβ ligand Derrière appears to signal similarly to Activin, and does not require EGF-CFC co-receptors (Chen et al., 2006; Cheng et al., 2003).

Although most, or indeed all, of the biological functions of Nodal ligands could reflect their ability to induce receptor-mediated responses, Nodal ligands have been shown to heterodimerize with other TGFβ superfamily members, such as Bmp4 and Derrière, to form signaling factors that have reduced or distinct activities (Eimon and Harland, 2002; Yeo and Whitman, 2001). Furthermore, Nodal, as well as Gdf3, can potentially inhibit BMPs as well as Wnt ligands via mechanisms that do not involve heterodimerization (Haramoto et al., 2004; Levine and Brivanlou, 2006; Onuma et al., 2005). To date, however, there is no definitive evidence supporting receptor-independent Nodal activity in vivo.

Receptors and co-receptors

Nodal ligands signal through the type I serine-threonine kinase receptor ALK4 (ActRIB/Acvr1b), together with the type II receptors ActRII (ActRIIA; Acvr2a) or ActRIIB (Acvr2b) (Reissmann et al., 2001; Yan et al., 2002; Yeo and Whitman, 2001). In contrast to Activin, Nodal ligands lack signaling activity in the absence of EGF-CFC co-receptors, despite their ability to interact with ALK4-ActRII complexes (Chen and Shen, 2004; Reissmann et al., 2001; Yeo and Whitman, 2001). The orphan type I receptor ALK7 (Acvr1c) can also transduce Nodal signaling activity, potentially in an EGF-CFC-independent manner (Reissmann et al., 2001); however, there is currently no evidence that ALK7 is required for Nodal pathway activity in vivo (Andersson et al., 2006; Jornvall et al., 2004).

Table 1. Key components of the Nodal signaling pathway

Role	Gene	Function	Key references
Pathway ligands	*Nodal* (mouse, chick), *cyclops, squint, southpaw* (fish), *Xnr1, Xnr2, Xnr4, Xnr5, Xnr6* (frog)	Nodal-related TGFβ ligands	(Brennan et al., 2001; Chen and Schier, 2001; Conlon et al., 1994; Feldman et al., 1998; Jones et al., 1995; Long et al., 2003; Lowe et al., 2001; Norris et al., 2002; Rebagliati et al., 1998b; Sampath et al., 1998; Vincent et al., 2003; Zhou et al., 1993)
	Vg1 (frog, fish, chick)	TGFβ ligand; signals through Nodal pathway	(Birsoy et al., 2006; Cheng et al., 2003)
	Gdf1 (mouse)	TGFβ ligand; signals through Nodal pathway	(Andersson et al., 2006; Cheng et al., 2003; Rankin et al., 2000)
	Gdf3 (mouse)	TGFβ ligand; signals through Nodal pathway	(Chen et al., 2006)
Receptors and co-receptors	*ALK4*	Type I serine-threonine kinase receptor	(Reissmann et al., 2001; Yan et al., 2002; Yeo and Whitman, 2001)
	ActRII, ActRIIB	Type II serine-threonine kinase receptors	(Reissmann et al., 2001; Song et al., 1999; Yan et al., 2002; Yeo and Whitman, 2001)
	Cripto, Cryptic (mouse), *one-eyed pinhead* (fish), *FRL-1/XCR1, XCR2, XCR3* (frog)	EGF-CFC co-receptors; interact with ALK4	(Branford and Yost, 2002; Ding et al., 1998; Dorey and Hill, 2006; Feldman et al., 2002; Gritsman et al., 1999; Onuma et al., 2006; Schier et al., 1997; Yan et al., 1999; Zhang et al., 1998)
Inhibitors	*Lefty1, Lefty2*	TGFβ proteins; interact with Nodal ligands and EGF-CFC co-receptors	(Chen and Shen, 2004; Chen and Schier, 2002; Cheng et al., 2004; Meno et al., 1999; Meno et al., 1998; Meno et al., 2001; Nakamura et al., 2006; Perea-Gomez et al., 2002)
	Cer1, Cer2	Cerberus/DAN family members; interact with Nodal ligands	(Bertocchini and Stern, 2002; Hashimoto et al., 2004; Marques et al., 2004; Perea-Gomez et al., 2002; Piccolo et al., 1999)
Smads	*Smad2, Smad3*	Receptor-Smads	(Dunn et al., 2004; Vincent et al., 2003)
	Smad4	Co-Smad	(Chu et al., 2004)
Transcription factors	*FoxH1*	Winged-helix transcription factor	(Germain et al., 2000; Hoodless et al., 2001; Kunwar et al., 2003; Pogoda et al., 2000; Saijoh et al., 2000; Sirotkin et al., 2000; Watanabe and Whitman, 1999; Yamamoto et al., 2001)
	Mixer	Homeodomain protein	(Germain et al., 2000; Hart et al., 2002; Kofron et al., 2004; Kunwar et al., 2003)

EGF-CFC proteins are essential co-receptors for Nodal that confer specificity for the type I receptor ALK4 through protein interactions (Yan et al., 2002; Yeo and Whitman, 2001). EGF-CFC genes represent a small family, with two members in mammals (*Cripto* and *Cryptic*), three in frogs (*FRL1/XCR1*, *XCR2*, and *XCR3*), and a single gene in zebrafish, *one-eyed pinhead* (*oep*) (Dorey and Hill, 2006; Shen and Schier, 2000) (Table1). Zebrafish embryos that lack both maternal and zygotic contributions of *oep* phenocopy double mutants for the Nodal ligands *cyc* and *sqt*, whereas the expression of Activin, but not Nodal, can rescue the *oep* phenotype (Gritsman et al., 1999). Notably, *oep* acts as a cis-acting permissive factor based on its cell-autonomy in chimeric zebrafish embryos that are generated by cell transplantation (Schier et al., 1997; Strahle et al., 1997), as well as its inability to induce phenotypes when overexpressed (Zhang et al., 1998). In certain contexts, however, EGF-CFC proteins can have distinct properties: mouse Cripto can act as a secreted trans-acting factor to mediate Nodal signaling in cell culture and in vivo (Chu et al., 2005; Yan et al., 2002), whereas soluble human Cripto protein (TDGFI) can activate the Ras/Raf/MAPK and PI3K/Akt pathways in a Nodal-independent manner in mammary epithelial cells (Bianco et al., 2002; Bianco et al., 2003). Furthermore, a recent study has reported that the non-canonical Wnt ligand Wnt11 specifies the dorsal-ventral axis in *Xenopus* embryos through an interaction with FRL1, leading to the activation of the canonical Wnt/β-catenin pathway (Tao et al., 2005).

Extracellular inhibitors

Multiple extracellular inhibitors can modulate the activity of Nodal ligands. The Lefty proteins, which are highly diverged members of the TGFβ superfamily, antagonize Nodal signaling through their interactions with EGF-CFC proteins as well as Nodal ligands, thereby blocking formation of receptor complexes (Chen and Shen, 2004; Cheng et al., 2004). By contrast, Lefty proteins have not been found to interact with ALK4 or ActRIIB, indicating that they do not function as competitive inhibitors of these receptors (Chen and Shen, 2004; Cheng et al., 2004). Notably, Lefty genes are often downstream targets of Nodal

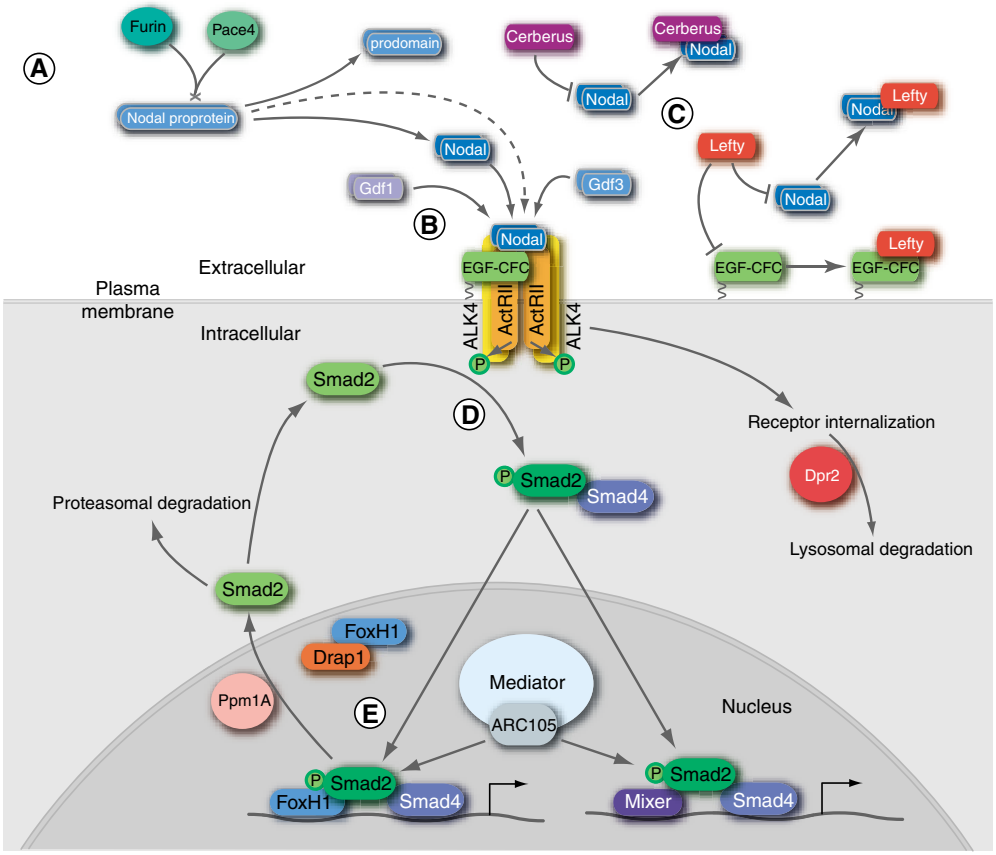

Fig. 1. Schematic outline of the Nodal signaling pathway. (**A**) Nodal ligands are expressed as homodimeric proproteins, and can be cleaved extracellularly by the proprotein convertases Furin and Pace4. (**B**) Mature Nodal ligands, as well as Gdf1 and Gdf3, can bind to an EGF-CFC co-receptor in a complex with type I receptor (ALK4) and type II receptor (ActRII or ActRIIB) dimers. At least in some contexts, uncleaved Nodal proprotein can also signal through a similar receptor complex, although it is currently unknown whether such signaling is EGF-CFC dependent (Ben-Haim et al., 2006). (**C**) Cerberus and Lefty proteins are soluble antagonists that can interact with Nodal ligands; Lefty proteins can also interact with EGF-CFC co-receptors to inhibit their function. (**D**) Receptor activation leads to the phosphorylation of the type I receptor by the type II kinase, as well as phosphorylation of Smad2 (or Smad3). Activated Smad2 or Smad3 associates with Smad4 and translocates to the nucleus, whereas the receptor complex undergoes internalization into endosomes and can be targeted by Dpr2 for lysosomal degradation. (**E**) Within the nucleus, activated Smad2-Smad4 (or Smad3-Smad4) complexes interact with the winged-helix transcription factor FoxH1 or Mixer homeoproteins on target promoters, leading to transcriptional activation through interactions with ARC105 and the Mediator complex. Pathway activity can be inhibited by interaction of Drap1 with FoxH1 or by the Smad phosphatase Ppm1A, which promotes the nuclear export of Smad2 and possibly targets it for proteasomal degradation.

signaling, forming an important negative-feedback mechanism for this pathway (Branford and Yost, 2002; Feldman et al., 2002; Meno et al., 1999).

Members of the Cerberus family are cysteine-rich extracellular proteins that can block Nodal signaling through their direct interactions with Nodal ligands (Piccolo et al., 1999). Despite the multifunctional ability of Cerberus to antagonize BMP and Wnt signaling in *Xenopus* (Silva et al., 2003), the mouse Cerberus proteins are primarily Nodal inhibitors (Marques et al., 2004; Perea-Gomez et al., 2002). In addition to secreted inhibitors of Nodal signaling, several membrane-associated proteins, such as Tomoregulin-1 (Tmeff1) and Nicalin, have been proposed to represent pathway antagonists (Haffner et al., 2004; Harms and Chang, 2003). However, the precise relationship of these membrane-associated inhibitors to Nodal function in vivo remains to be clarified.

Signal transducers and transcriptional regulators

Downstream of ALK4 and ActRII receptors, Nodal pathway activity is transduced by the receptor-associated Smads (R-Smads) Smad2 and/or Smad3, together with the common mediator-Smad (co-Smad) Smad4 (Massague et al., 2005). Although Smad2 and Smad3 have differing abilities to regulate target gene transcription, gene substitution experiments in the mouse have shown that *Smad3* can functionally replace *Smad2* in vivo (Dunn et al., 2005). Surprisingly, the phenotype of *Smad4*-null mutants is significantly less severe than that of *Nodal* mutants or *Smad2; Smad3* double mutants (Chu et al., 2004), suggesting that other proteins may provide a co-Smad function in early mouse development. Such a possibility is consistent with the ability of the nuclear protein TIF1γ (Trim33 – Mouse Genome Informatics) to interact with activated Smad2/3 to mediate TGFβ signaling in hematopoietic progenitors (He et al., 2006).

At the transcriptional level, Nodal pathway function is tightly coupled with the activities of the winged-helix transcription factor FoxH1 and the Mixer subclass of homeodomain proteins. These proteins contain Smad-interaction motifs that are required for their interaction with Smad2/Smad3, leading to the formation of active transcription complexes on the enhancers of Nodal pathway target genes (Germain et al., 2000; Randall et al., 2004). However, genetic analyses in zebrafish indicate that FoxH1 and Mixer do not fully

Box 1. The identification of Nodal ligands

Unlike most other key developmental regulators, *Nodal* was isolated from a retroviral insertional mutagenesis screen in mouse embryonic stem cells (Robertson et al., 1986). This screen led to the discovery of a retrovirally-induced mutant that displayed an early gastrulation-defective phenotype (Conlon et al., 1991; Conlon et al., 1994). The corresponding locus was subsequently shown to encode a member of the TGFβ ligand superfamily that is expressed in the mammalian node (Zhou et al., 1993). The identification of *Nodal*-related ligands in other species followed rapidly (Jones et al., 1995; Levin et al., 1995; Rebagliati et al., 1998a). In parallel, genetic screens in the zebrafish isolated loss-of-function alleles in two Nodal-related genes, *cyclops* (*cyc*) and *squint* (*sqt*), and demonstrated their essential roles in gastrulation (Feldman et al., 1998; Rebagliati et al., 1998b; Sampath et al., 1998). Much subsequent work has led to the realization that Nodal pathway activity is responsible for many of the biological functions previously attributed to other TGFβ superfamily members, particularly Activin (Schier and Shen, 2000).

Box 2. Processing and signaling ability of Nodal ligands

TGFβ ligand proproteins undergo dimerization, which is facilitated by an intrachain disulfide bond; interestingly, the cysteine residue involved is absent in Lefty and Gdf3 proteins, suggesting that these proteins are either monomers or relatively labile dimers. The cleavage of proprotein dimers has been thought to occur intracellularly in the trans-Golgi network. Following proprotein cleavage, dimeric TGFβ prodomains often remain non-covalently associated with the mature dimeric ligand, together with Latent TGFβ binding proteins (LTBPs). This maintains the mature ligand in a biologically inactive state, and an activation step is required to release the mature ligand from the latent complex.

In the case of Nodal, however, processing by the proprotein convertases Furin and Pace4 can occur extracellularly, rather than in the trans-Golgi network, consistent with their non-cell-autonomous role in pre-gastrulation mouse embryos (Beck et al., 2002). Moreover, it is currently unknown whether Nodal ligands exist in a latent complex or are regulated in a similar fashion. In addition, although most TGFβ ligands lack signaling activity as proproteins, mutant Xnr2 and Nodal proproteins that cannot be cleaved can retain activity in vivo (Ben-Haim et al., 2006; Eimon and Harland, 2002).

account for Nodal-mediated transcriptional events (Kunwar et al., 2003), indicating that additional transcription factors involved in Nodal responses remain to be identified.

At present, most known targets of the Nodal pathway, such as *Nodal*, *Lefty2*, *Pitx2*, *FoxA2* and *Lhx1* (Table 1), undergo transcriptional activation in response to Nodal signals, whereas a few are transcriptionally repressed (Dickmeis et al., 2001; Whitman, 2001). *Nodal* itself is positively autoregulated through the asymmetric enhancer (ASE) located in its first intron (Adachi et al., 1999; Norris et al., 2002; Norris and Robertson, 1999), and by an upstream left-side specific enhancer (LSE) (Saijoh et al., 2005; Vincent et al., 2004). In part, transcriptional activation is likely to occur through the interaction of Smad2/3-Smad4 proteins with ARC105, a subunit of the Mediator transcriptional co-activator complex (Kato et al., 2002). Transcriptional activation is also likely to require chromatin remodelling mediated by the ability of phosphorylated Smad2 to recruit the p300 histone acetyltransferase as well as Brg1 (Smarca4 – Mouse Genome Informatics), a component of the SWI/SNF chromatin remodelling complex (He et al., 2006; Ross et al., 2006).

The generation and interpretation of graded Nodal signals

Nodal ligands have the properties associated with a morphogen: a signal that acts over a distance to elicit dose-dependent responses in a developmental field of responsive cells (Ashe and Briscoe, 2006). The mechanisms by which such graded signals can be generated and interpreted have been of particular interest because they are fundamental for embryonic tissue patterning.

Long-range action

In zebrafish, the Nodal ligand Sqt as well as its inhibitor Lefty can function as long-range mesoderm-inducing signals in vivo, whereas Cyc cannot (Chen and Schier, 2001; Chen and Schier, 2002). Cell-transplantation experiments in zebrafish have shown that Sqt signals can traverse cells that lack the EGF-CFC co-receptor Oep, and thus are unresponsive to Sqt, to activate responses in distant wild-type cells in the absence of a signaling relay mechanism (Chen and Schier, 2001). In the mouse, *Lefty2* hypomorphic mutants display ectopic Nodal pathway activation in the right lateral plate mesoderm, suggesting that an excess of left-sided Nodal protein has undergone long-range diffusion to the right side (Meno et al., 2001). Consistent with these findings, GFP-labeled Nodal or Lefty2 proteins can travel over long distances (up to 500 μm) when expressed in chick embryos (Sakuma et al., 2002). Furthermore, the visualization of GFP-tagged Xnr2 protein movement from Xnr2-expressing *Xenopus* animal caps into adjacent non-expressing caps has revealed no evidence of transcytosis, argosomal transport or cytonemes; instead, long-range movement of Xnr2 appears to occur by diffusion through the extracellular matrix (Williams et al., 2004).

Recent findings suggest that the stability as well as the efficiency of Nodal ligand processing are primary determinants of their signaling range (Box 2). Studies in cell culture and in zebrafish have shown that the Nodal proprotein is relatively stable, whereas the processed mature ligand is readily degraded following its cellular internalization (Le Good et al., 2005). In particular, the long-range movement of Nodal ligands may correspond to the diffusion of a relatively stable proprotein, and its subsequent extracellular cleavage by the proprotein convertases Furin (Spc1) or Pace4 (Spc4/Pcsk6) then generates a labile mature ligand (Beck et al., 2002; Le Good et al., 2005).

Dose-dependent responses

As is the case with other potent developmental signaling factors, Nodal signaling can induce dose-dependence in cellular responses. This is exemplified by Nodal-mediated specification of mesodermal identity, as initially shown in gain-of-function studies of Activin signaling in *Xenopus* (Green and Smith, 1990; Gurdon et al., 1994; Gurdon et al., 1999). Subsequent loss-of-function analyses of *sqt* and *cyc* double mutants in fish have demonstrated that different levels of Nodal activity are required for the patterning of the mesoderm along the animal-vegetal axis (Dougan et al., 2003), including the specification of prechordal mesoderm versus notochord during axial mesoderm differentiation (Gritsman et al., 2000). A similar dose-dependent response to Nodal pathway activity in mice has been supported by the progressively more severe defects in mesendoderm formation observed in increasing doses of *Smad2* and *Smad3* mutant alleles (Dunn et al., 2004; Vincent et al., 2003).

The dose-dependent responses of cells to Nodal signaling may be due to differing levels of Nodal pathway activity, or to differing durations of exposure, or both (Gritsman et al., 2000). Responding cells appear to be exquisitely sensitive to Activin/Nodal levels, even without amplification of pathway activity, as threefold differences

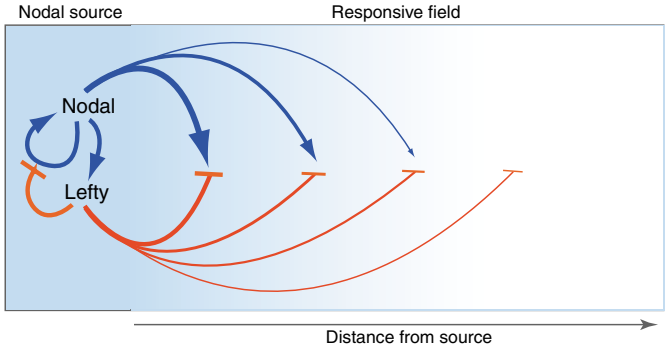

Fig. 2. Reaction-diffusion mechanism for the generation of positional information. The generation of a stable Nodal signaling gradient (shown in blue) across a developmental field can result from a source of Nodal signals (at left) that undergo positive autoregulation and act at long-range (blue arrows). The expression of Lefty inhibitor (red) is also induced by the Nodal pathway, and has a greater range than Nodal signals. Cells in close proximity to the Nodal source thereby perceive high levels of signaling activity, whereas more distant cells perceive little or no signaling activity, as lateral inhibition by Lefty will prevail over a longer range. Such a regulatory mechanism for Nodal pathway activity may function during mesendoderm specification and left-right patterning. [Adapted from Branford and Yost (Branford and Yost, 2004).]

Box 3. The evolution of Nodal pathway function

Key components of the Nodal pathway appear to be absent from protostomes, as suggested by the absence of Nodal, EGF-CFC, or Lefty orthologs in the *Drosophila* and *C. elegans* genomes. By contrast, Nodal and Lefty orthologs have been identified in cephalochordates, tunicates and echinoderms, indicating that the pathway is not restricted to vertebrates, but may be more broadly conserved in deuterostomes (Chea et al., 2005; Duboc and Lepage, 2006). [Protostomes and deuterostomes correspond to the two major groupings of bilaterian animals, and differ in whether the blastopore opening results in the formation of the mouth (prostostomes) or the anus (deuterostomes).] In particular, a *Nodal-Lefty-Pitx2* gene expression cassette is asymmetrically expressed in sea urchin embryos, highlighting its evolutionarily conserved role in deuterostomes (Duboc et al., 2005). Unexpectedly, however, this cassette is specifically expressed on the right side, not the left (Duboc et al., 2005). At earlier stages of development, Nodal signaling plays a central role in establishing the oral-aboral axis in sea urchins, although it is not required for mesoderm formation (Duboc et al., 2004; Flowers et al., 2004). Interestingly, the restricted expression of *Nodal* to the prospective oral ectoderm and its fundamental role in oral-aboral axis specification has led to the speculation that the Nodal pathway arose in deuterostomes to define the region of the mouth (Chea et al., 2005; Duboc and Lepage, 2006).

in receptor occupancy result in equivalent increases in nuclear Smad2 concentration (Shimizu and Gurdon, 1999). Importantly, overall pathway activity corresponds to the maximal level of receptor occupancy and Smad2 activation, which can be maintained even after ligand withdrawal (Bourillot et al., 2002). At present, the molecular mechanisms by which these differing levels of pathway activity are interpreted as distinct transcriptional responses remain to be elucidated.

Intracellular downregulation of pathway activity

Many potential mechanisms for downregulating intracellular pathway activity in Nodal-responsive cells have been described, although it is not yet understood how these might shape dose-dependent responses. For example, Dpr2 (Dapper2; Dact2 – Zebrafish Information Network), which was initially identified as a regulator of Wnt signaling, functions as an antagonist of Activin/Nodal pathway activity during zebrafish mesoderm formation by binding to endocytosed ALK4/ALK5 receptors and facilitating their degradation (Zhang et al., 2004). Recent work has also shown that the nuclear serine-threonine phosphatase Ppm1A can dephosphorylate activated Smad2 and Smad3, and can downregulate endogenous Nodal signaling activity in zebrafish embryos (Lin et al., 2006). At the transcriptional level, the general transcription factor Drap1 can dampen Nodal signaling in mouse embryos through an interaction with FoxH1 that inhibits its DNA-binding ability (Iratni et al., 2002). Finally, the loss of competence to respond to Activin/Nodal signals towards the end of mesoderm formation in *Xenopus* is associated with the exclusion of Smad2 from the nucleus (Grimm and Gurdon, 2002).

Reaction-diffusion mechanism

The regulatory properties of the Nodal pathway strongly resemble the characteristics of a biological reaction-diffusion system, which can generate a stable graded signal across a responsive developmental field (Fig. 2) (Chen and Schier, 2002; Saijoh et al., 2000). Such a reaction-diffusion system depends on the ability of ligands and antagonists to diffuse over a long distance, coupled with positive and negative autoregulatory loops (Meinhardt and Gierer, 2000). In particular, the diffusion of Lefty inhibitors in tissue appears to be more efficient than that of Nodal (Sakuma et al., 2002), which represents a crucial component of such a reaction-diffusion system. This mechanism is likely to function during mesoderm patterning, as well as in left-right specification (Chen and Schier, 2002; Nakamura et al., 2006).

Central functions of Nodal signaling in embryogenesis

Numerous biological activities in early embryogenesis have been ascribed to functions of the Nodal pathway. However, the roles of Nodal signaling and antagonism in mesoderm and endoderm induction, neural patterning and left-right specification appear to be particularly well-conserved (Box 3).

Mesoderm induction and patterning

Although Nodal pathway activity is essential for mesoderm formation, there appear to be species-specific differences in the relative roles of Nodal and Vg1/Gdf3 in this process, and in their interactions with the canonical Wnt signaling pathway. In *Xenopus* embryos, the maternally-encoded VegT transcription factor cooperates with activated β-catenin to activate zygotic transcription of Xnr and Vg1 ligands in the vegetal region, leading to a dorsal-ventral (D-V) graded Nodal signal that induces dose-dependent mesendoderm formation in the marginal zone, with higher levels resulting in dorsal specification (Fig. 3A) (Agius et al., 2000; Kimelman, 2006). Alternatively, D-V mesoderm patterning might be due to differences in the timing of the onset of Nodal signaling, with earlier and longer signaling leading to a dorsal identity (Lee et al., 2001).

The expression of zygotic *sqt* and *cyc* in the zebrafish embryo is induced by an as yet unidentified β-catenin-dependent signal(s) in the extraembryonic yolk syncytial layer (YSL) (Chen and Kimelman, 2000), resulting in mesendoderm formation at the

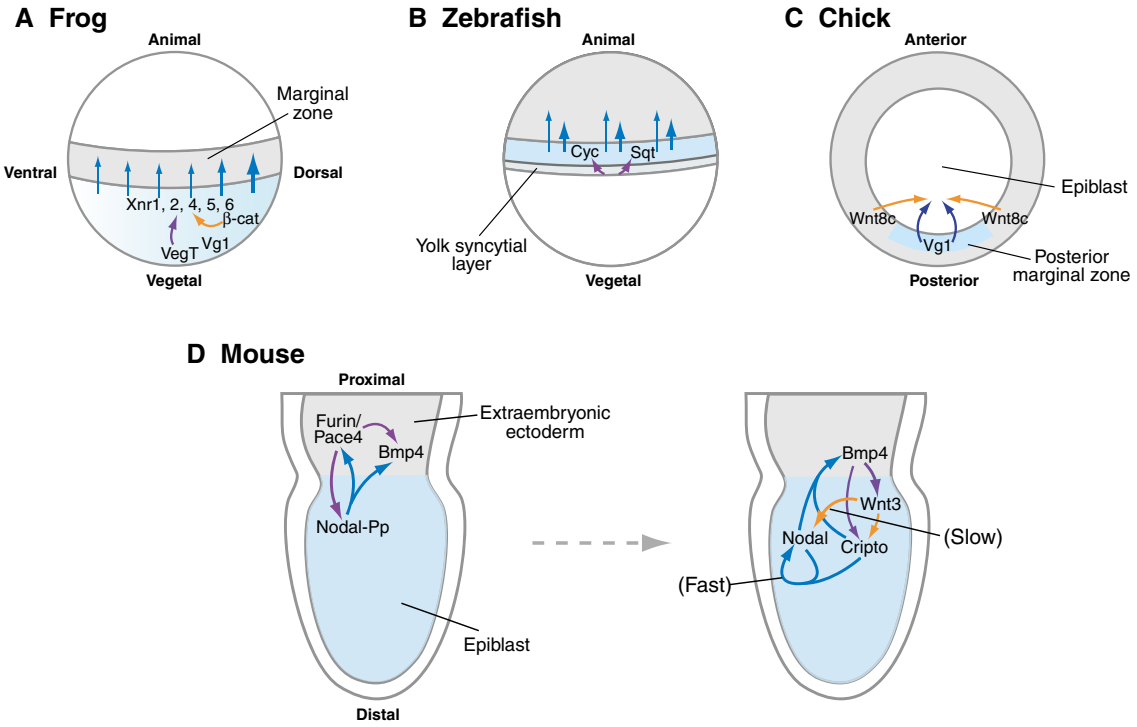

Fig. 3. Models of regulatory pathways for mesoderm induction. Depictions of embryos at pre-gastrulation stages. Domains of *Nodal/Vg1* expression are indicated in blue; blue arrows indicate *Nodal/Vg1* activity, orange arrows indicate Wnt/β-catenin activity, and purple arrows correspond to the activity of other factors as noted. (**A**) In *Xenopus* (lateral view), zygotic *Xnr* transcripts (blue arrows) are activated by the maternally encoded VegT T-box transcription factor (purple arrow). Cortical rotation after fertilization leads to translocation of maternal dorsalizing signals and the stabilization of β-catenin (orange arrow) on the dorsal side. The levels of *Xnr* as well as maternal *Vg1* transcripts are higher dorsally (thicker blue arrows), and specify the dorsal-ventral patterning of the mesoderm in the marginal zone. (**B**) In zebrafish (lateral view), zygotic *cyc* and *sqt* transcripts (blue arrows) at the blastoderm margin are activated by an as yet unidentified signal(s) that emanates from the extraembryonic yolk syncytial layer. Graded Nodal signaling (thin and thick arrows) specifies the animal-vegetal patterning of mesoderm. (**C**) In the chick embryo (dorsal view), *Vg1* (blue arrow) expressed at the posterior marginal zone cooperates with posteriorly-expressed *Wnt8c* (orange arrow) to induce streak formation in the adjacent epiblast. (**D**) In the mouse embryo (lateral view), Nodal proprotein (Nodal-Pp) expressed in the epiblast signals to the extraembryonic ectoderm, which activates expression of its proprotein convertases Furin and Pace4, as well as Bmp4. Production of the active mature Nodal ligand induces its positive autoregulatory loop (fast-acting; blue arrow), as well as a slower feedback loop (orange arrow) through Bmp4 and Wnt3; an additional feedback loop may take place through Cripto upregulation by Bmp4 and Wnt3 (Beck et al., 2002; Morkel et al., 2003).

blastoderm margin (Fig. 3B). Interestingly, genetic analyses in zebrafish have suggested that long-range graded Nodal signaling is responsible for mesoderm patterning along the animal-vegetal axis, not along the D-V axis (Dougan et al., 2003). Finally, in the chick embryo, Vg1 plays a primary role together with Wnt8c to induce primitive streak formation in the posterior marginal zone, and subsequently induce *Nodal* expression in the epiblast (Fig. 3C) (Bertocchini et al., 2004; Skromne and Stern, 2001).

A more complex regulatory circuit that utilizes interlinked fast and slow positive regulatory loops is employed for primitive streak formation in the mouse embryo (Fig. 3D). The analysis of a Nodal prodomain cleavage mutant has indicated that unprocessed Nodal ligand can signal from the epiblast to the adjacent extraembryonic ectoderm to induce the expression of Furin and Pace4 proprotein convertases as well as Bmp4 (Ben-Haim et al., 2006). Subsequently, Bmp4 signals back to the epiblast to activate *Wnt3* expression, which can upregulate *Nodal* and *Cripto* expression in the epiblast through the canonical Wnt pathway (Ben-Haim et al., 2006; Morkel et al., 2003). Furthermore, Gdf3 is also likely to function in these feedback loops, as *Gdf3*-null mutants display variable defects in the mesoderm and definitive endoderm that correlate with altered *Nodal* expression levels (Chen et al., 2006).

Endoderm formation

The formation of the endoderm also requires Nodal signaling, which is mediated by Mixer homeoproteins (Lewis and Tam, 2006; Stainier, 2002), and may represent a dose-dependent response to levels of Nodal activity that are higher than those required for mesoderm formation (Agius et al., 2000; Thisse et al., 2000; Vincent et al., 2003). In *Xenopus*, endoderm formation can be induced by overexpression of four of the seven Mixer (Mix/Bix) -related homeoproteins, and can be abolished by morpholino knock-down of *Mixer* (*Mix.3*) (Kofron et al., 2004). Conversely, knock-down of *Mixer* expands the mesoderm, as shown by the upregulation of *Xnr1* and *Xnr5*, and increases mesoderm-inducing activity in animal cap assays (Kofron et al., 2004). Similarly, null mutants for the mouse *Mixl1* (*Mml*) homeobox gene display reduced definitive endoderm, but also overexpress *Nodal* and generate excess axial mesoderm (Hart et al., 2002). Taken together, these studies suggest that Mixer homeoproteins are expressed in mesendoderm progenitors and specify endoderm in response to high-level Nodal signals by inducing endoderm-specific genes such as *Sox17*, while simultaneously repressing the expression of several mesoderm-inducing genes, including those encoding Nodal ligands.

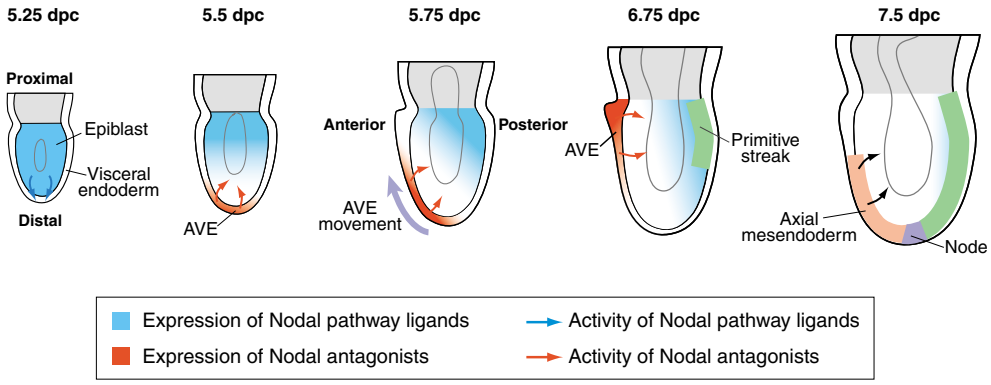

Fig. 4. Anterior neural patterning by Nodal signaling and antagonism in the mouse embryo. Blue shading indicates regions expressing *Nodal* and/or *Gdf3*; red shading indicates regions expressing the Nodal antagonists *Lefty1* and *Cer1*. Shortly after implantation, *Nodal* is expressed throughout the epiblast [5.25 days post-coitum (dpc)], and induces formation of the anterior visceral endoderm (AVE; red) at the distal end of the egg cylinder at 5.5 dpc; note that the initial appearance of the AVE is already slightly asymmetric, with a bias towards the prospective anterior side (Yamamoto et al., 2004). Nodal signaling is also required for the movement of the AVE (purple arrow) to the anterior side (5.75 dpc), where the expression of Nodal antagonists (*Lefty1*, *Cer1*) by the AVE is essential for the specification of anterior neural identity in the adjacent epiblast. Conversely, Nodal signaling is required for the generation of axial mesendoderm (orange) by the anterior primitive streak during gastrulation (7.5 dpc); in turn, the axial mesendoderm produces signaling factors (black arrows) that are essential for forebrain maintenance and ventral neural tube patterning.

Neural patterning

Nodal signaling plays dual roles in neural development, as the generation of anterior neural tissue requires its inhibition, whereas the subsequent maintenance and patterning of neural tissue depends upon axial mesendoderm generated in response to Nodal signaling. In the mouse embryo, the anterior visceral endoderm (AVE) plays a key role in anterior specification of the adjacent epiblast (Fig. 4) (Rossant and Tam, 2004). The AVE produces the Nodal antagonists Lefty1 and Cerberus-1 (Cer1), which are essential for anterior neural patterning and which prevent the formation of enlarged or duplicated primitive streaks that result from excessive Nodal activity (Perea-Gomez et al., 2002). Similarly, in the chick embryo, *Cerberus* expressed in the extraembryonic hypoblast (analogous to the mouse AVE) positions and limits the primitive streak, in cooperation with another Nodal antagonist that may be *Lefty1* (Bertocchini et al., 2004; Bertocchini and Stern, 2002). Furthermore, *Cerberus* expressed in the anterior endoderm antagonizes Nodal and Wnt ligands to allow head formation in *Xenopus* (Piccolo et al., 1999).

By contrast, high levels of Nodal activity in the posterior epiblast are required for the generation of the prechordal mesoderm and anterior endoderm (Vincent et al., 2003), which are necessary in turn for ventral patterning of the neural tube and the maintenance of anterior forebrain territories (Fig. 4). Consequently, reductions of Nodal pathway activity can result in phenotypes that resemble human holoprosencephaly, as observed in zygotic *oep* zebrafish mutants or hypomorphic *Cripto* mouse mutants (Chu et al., 2005; Schier et al., 1997). Furthermore, analyses of human holoprosencephaly patients have identified genetic loci that include regulators of the Nodal pathway, such as *Cripto* and the transcriptional repressor *TGIF* (de la Cruz et al., 2002; Gripp et al., 2000).

Left-right patterning

During left-right (L-R) axis specification, Nodal pathway activity regulates the propagation of left-sided positional information from the node to the left lateral plate mesoderm (LPM), and is required in both locations (Raya and Belmonte, 2006; Shiratori and Hamada, 2006). Following initial events that establish L-R asymmetry, Nodal activity is upregulated on the left side of the node (Fig. 5A), through a process that might involve asymmetric Ca^{2+} signaling and Notch pathway activity. It is known that the asymmetric expression of *Nodal* occurs in the left LPM of all vertebrate species thus far examined, and leads to tissue-specific laterality decisions.

In the mouse, *Nodal* expression in the node is essential for the subsequent asymmetric gene expression in the LPM (Brennan et al., 2002; Saijoh et al., 2003). In zebrafish, however, expression of the Nodal ligand *spaw* around Kupffer's vesicle is not essential for *spaw* expression in the LPM (Long et al., 2003), suggesting that a different factor is involved in the transfer of left-sided information. Intriguingly, mouse *Gdf1* is expressed in the peri-nodal region as well as in the LPM (Wall et al., 2000), and *Gdf1*-null mutants have a L-R patterning phenotype indistinguishable from that of mutants for the EGF-CFC gene *Cryptic* (Rankin et al., 2000; Yan et al., 1999). A role for Nodal pathway function in the node is further supported by studies of zebrafish *charon* and mouse *Cer2* (*Dante*, *Dand5*), genes that encode Cer/DAN family members that can antagonize Nodal ligands (Hashimoto et al., 2004; Marques et al., 2004).

Current models suggest that Nodal and/or Gdf1 proteins signal at long-range from the node to the LPM, although a signal relay mechanism involving intermediary signaling factors has not been excluded. In the mouse, evidence supporting a direct signaling interaction has emerged from studies of *Nodal* promoter elements, in particular the left-side enhancer (LSE) and asymmetric enhancer (ASE), which both drive *Nodal* expression in the left LPM, and contain FoxH1-binding sites that are essential for their function (Norris et al., 2002; Saijoh et al., 2000; Saijoh et al., 2005; Vincent et al., 2004). The subsequent auto-activation of *Nodal* results in the rapid spread of *Nodal* expression throughout the left LPM, as well as the induction of *Pitx2* expression and the subsequent downregulation of Nodal activity by *Lefty2* via a negative-feedback loop (Fig. 5B). Nodal signaling is also essential for the expression of *Lefty1* in the axial midline, which can act as a molecular barrier that prevents the leakage of left-sided Nodal signals to the right side, and can suppress ectopic right-sided activity (Meno et al., 1998; Yamamoto et al., 2003). Mathematical modeling shows that this

Nodal-Lefty-Pitx2 expression cassette generates a modified reaction-diffusion mechanism that ensures the uniform propagation of Nodal signals throughout the left LPM, while inhibiting its spread to the right side (Nakamura et al., 2006).

Novel functions of the Nodal pathway

Over recent years, unexpected roles for Nodal signaling have continued to emerge. The extent to which these functions are evolutionarily conserved is currently unknown.

Dorsal-ventral axis specification by maternal transcripts

Although the activation of canonical Wnt signaling specifies dorsal identity in frogs and zebrafish (Kimelman, 2006; Schier and Talbot, 2005), a recent study has proposed that maternal transcripts for the Nodal ligand *sqt* act as dorsal determinants in zebrafish (Gore et al., 2005). In particular, maternal *sqt* transcripts are localized in dorsal blastomeres in a majority of zebrafish embryos at the four-cell and eight-cell stages, whereas morpholino knock-down of maternal *sqt* leads to a ventralized phenotype (Gore et al., 2005). The localization of *sqt* transcripts to dorsal blastomeres is conferred by sequence motifs in the 3′ untranslated region (UTR), which can confer a similar localization when fused in cis to heterologous *lacZ* mRNA (Gore et al., 2005); the underlying mechanism may resemble those utilized for active transport of mRNA transcripts in *Drosophila* and *Xenopus* embryos (Palacios and St Johnston, 2001). Interestingly, these sequence motifs also occur in *Nodal* genes in several mammalian species, including human, raising the possibility of a conserved developmental mechanism (Gore et al., 2005). However, the significance of these findings is currently uncertain because the phenotype of maternal-zygotic *sqt* mutants resembles that of zygotic *sqt* mutants (Aoki et al., 2002), suggesting that maternal *sqt* transcripts are not essential for dorsal specification.

Anterior-posterior axis formation

In the mouse, Nodal signaling is required for at least two events associated with anterior-posterior (A-P) axis specification: the formation and directional movement of the AVE (Fig. 4). The AVE initially forms in the most distal portion of the post-implantation egg cylinder, but then translocates to the prospective anterior side within 12 hours (Rivera-Perez et al., 2003; Srinivas et al., 2004; Thomas et al., 1998). In the absence of *Nodal*, no AVE is formed and no evidence of an A-P axis is apparent (Brennan et al., 2001; Norris et al., 2002). In the absence of *Cripto* or in hypomorphic *Nodal* mutants, the AVE forms but does not translocate (Ding et al., 1998; Lowe et al., 2001; Norris et al., 2002). The activity of the mouse Nodal pathway ligand Gdf3 is also crucial for AVE induction, as well as for its movement, as both processes can be affected in *Gdf3*-null mutants (Chen et al., 2006). Finally, Nodal activity might also play an indirect permissive role in these processes, as AVE formation may require the epiblast to reach a threshold size to dilute an inhibitory signal from the distant extraembryonic ectoderm (Rodriguez et al., 2005), a process that is impaired in *Nodal* mutants (Mesnard et al., 2006).

Prior to AVE movement, the expression of *Lefty1* and *Cer1* in the distal visceral endoderm displays a slightly asymmetric bias toward the prospective anterior side (Takaoka et al., 2006; Yamamoto et al., 2004). This asymmetric expression of Nodal antagonists has been proposed to mediate directional AVE movement by inhibiting cell proliferation in the visceral endoderm on the prospective anterior side, while allowing Nodal activity to drive cell proliferation posteriorly (Yamamoto et al., 2004). Interestingly, A-P polarity may exist at even earlier stages, as the asymmetric expression of a *Lefty1-lacZ* transgene can be detected in the primitive endoderm of the peri-implantation embryo (Takaoka et al., 2006). Although *Lefty1* itself is not required for A-P axis formation, expression of the *Lefty1-lacZ* transgene is abolished in *FoxH1* mutants (Takaoka et al., 2006), suggesting that Nodal pathway function is essential for early A-P polarity in the mouse.

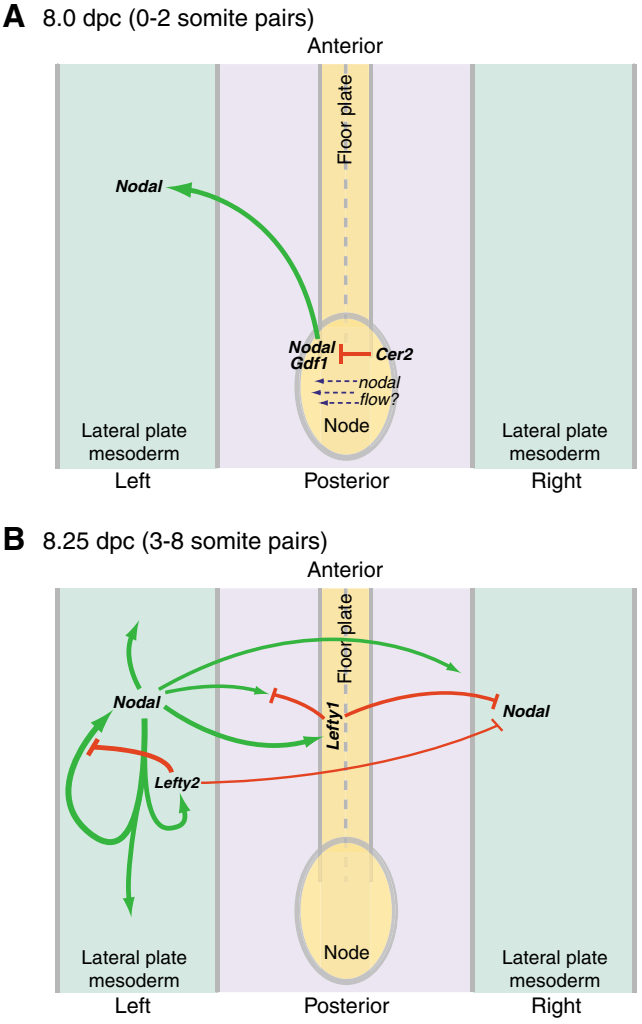

Fig. 5. Sequential function of Nodal signaling in left-right patterning in the mouse embryo. (**A**) Following initial symmetry breaking around the node, possibly as a consequence of ciliary-based nodal flow, Nodal (green arrow) and/or Gdf1 signals become elevated on the left side of the node, and are antagonized by Cer2 (red). Nodal pathway activity then propagates to the left lateral plate mesoderm to activate left-sided *Nodal* expression, most likely through direct long-range action. (**B**) *Nodal* auto-regulates its own expression, which spreads through the left lateral plate mesoderm (green) through a positive-feedback loop. *Lefty2* is induced through a negative-feedback loop, and subsequently downregulates *Nodal* expression (red bar). Axial midline expression of *Lefty1* prevents the spread of left-sided *Nodal* signals, and suppresses ectopic *Nodal* activation on the right side.

Maintenance of undifferentiated ES cells

Recent studies have suggested that Nodal signaling is required for the maintenance of undifferentiated human and mouse embryonic stem (ES) cells. Indeed, all key components of the Nodal pathway are highly expressed in both undifferentiated mouse and human ES

cells (Brandenberger et al., 2004). Overexpression of Nodal in human ES cells inhibits mesoderm differentiation within embryoid bodies (formed from three-dimensional aggregates of ES cells in culture), and maintains cells in the undifferentiated state, while simultaneously promoting visceral endoderm differentiation at the surface of embryoid bodies (Vallier et al., 2004). Conversely, the inhibition of pathway activity leads to decreased stem cell self-renewal and loss of expression of the pluripotency regulators *OCT4* (*POU5F1* – Human Gene Nomenclature Database) and *NANOG* (James et al., 2005; Vallier et al., 2005). By contrast, similar treatment of mouse ES cells does not yield the same effects, suggesting possible species-specific differences in Nodal function (James et al., 2005). However, these observations are consistent with findings that Nodal signaling in vivo is required to maintain epiblast pluripotency and prevent precocious neural differentiation (Brennan et al., 2001; Camus et al., 2006; Ding et al., 1998; Mesnard et al., 2006).

Potential role in carcinogenesis

Although most genes in the Nodal pathway are rarely expressed during later development and adulthood, there is evidence that pathway activity is upregulated in many human cancers. In particular, increased expression of *Nodal* in malignant melanoma is correlated with cancer progression, whereas pathway inhibition decreases tumorigenicity in xenograft assays (Topczewska et al., 2006). These findings are consistent with the upregulation of *Cripto* that is observed in many epithelial cancers (Strizzi et al., 2005), and with the ability of *Cripto* overexpression to promote tumorigenesis in xenografts and transgenic mice (Adkins et al., 2003; Sun et al., 2005). The mechanisms by which Nodal signaling may facilitate cancer progression remain unclear, but analyses of transgenic mice have suggested that *Cripto* can induce an epithelial-mesenchymal transition (Strizzi et al., 2004). At present, however, it remains unknown whether any of the oncogenic effects of *Cripto* are dependent on Nodal pathway activity.

Conclusion

Despite two decades of study of the Nodal pathway, numerous important questions regarding its functions and molecular mechanisms remain unanswered. Given the close apposition of distinct pathway functions in space and time, future studies will undoubtedly employ precise genetic tools to remove pathway activity in specific tissues and/or developmental stages. Furthermore, the cross-talk between this pathway and the parallel and/or synergistic functions of the canonical Wnt pathway will require additional investigation. Finally, the potential roles of Nodal pathway components in stem cell pluripotency and cancer progression will propel further studies of their function, and may provide future therapeutic targets.

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References

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 lefty-2 enhancer. *Genes Dev.* **13**, 1589-1600.

Adkins, H. B., Bianco, C., Schiffer, S. G., Rayhorn, P., Zafari, M., Cheung, A. E., Orozco, O., Olson, D., De Luca, A., Chen, L. L. et al. (2003). Antibody blockade of the Cripto CFC domain suppresses tumor cell growth in vivo. *J. Clin. Invest.* **112**, 575-587.

Agius, E., Oelgeschlager, M., Wessely, O., Kemp, C. and De Robertis, E. M. (2000). Endodermal Nodal-related signals and mesoderm induction in Xenopus. *Development* **127**, 1173-1183.

Andersson, O., Reissmann, E., Jornvall, H. and Ibanez, C. F. (2006). Synergistic interaction between Gdf1 and Nodal during anterior axis development. *Dev. Biol.* **293**, 370-381.

Aoki, T. O., Mathieu, J., Saint-Etienne, L., Rebagliati, M. R., Peyrieras, N. and Rosa, F. M. (2002). Regulation of nodal signalling and mesendoderm formation by TARAM-A, a TGFbeta-related type I receptor. *Dev. Biol.* **241**, 273-288.

Ashe, H. L. and Briscoe, J. (2006). The interpretation of morphogen gradients. *Development* **133**, 385-394.

Beck, S., Le Good, J. A., Guzman, M., Haim, N. B., Roy, K., Beermann, F. and Constam, D. B. (2002). Extraembryonic proteases regulate Nodal signalling during gastrulation. *Nat. Cell Biol.* **4**, 981-985.

Ben-Haim, N., Lu, C., Guzman-Ayala, M., Pescatore, L., Mesnard, D., Bischofberger, M., Naef, F., Robertson, E. J. and Constam, D. B. (2006). The Nodal precursor acting via activin receptors induces mesoderm by maintaining a source of its convertases and BMP4. *Dev. Cell* **11**, 313-323.

Bertocchini, F. and Stern, C. D. (2002). The hypoblast of the chick embryo positions the primitive streak by antagonizing nodal signaling. *Dev. Cell* **3**, 735-744.

Bertocchini, F., Skromne, I., Wolpert, L. and Stern, C. D. (2004). Determination of embryonic polarity in a regulative system: evidence for endogenous inhibitors acting sequentially during primitive streak formation in the chick embryo. *Development* **131**, 3381-3390.

Bianco, C., Adkins, H. B., Wechselberger, C., Seno, M., Normanno, N., De Luca, A., Sun, Y., Khan, N., Kenney, N., Ebert, A. et al. (2002). Cripto-1 activates Nodal- and ALK4-dependent and -independent signaling pathways in mammary epithelial cells. *Mol. Cell. Biol.* **22**, 2586-2597.

Bianco, C., Strizzi, L., Rehman, A., Normanno, N., Wechselberger, C., Sun, Y., Khan, N., Hirota, M., Adkins, H., Williams, K. et al. (2003). A Nodal- and ALK4-independent signaling pathway activated by Cripto-1 through Glypican-1 and c-Src. *Cancer Res.* **63**, 1192-1197.

Birsoy, B., Kofron, M., Schaible, K., Wylie, C. and Heasman, J. (2006). Vg 1 is an essential signaling molecule in Xenopus development. *Development* **133**, 15-20.

Bourillot, P. Y., Garrett, N. and Gurdon, J. B. (2002). A changing morphogen gradient is interpreted by continuous transduction flow. *Development* **129**, 2167-2180.

Brandenberger, R., Wei, H., Zhang, S., Lei, S., Murage, J., Fisk, G. J., Li, Y., Xu, C., Fang, R., Guegler, K. et al. (2004). Transcriptome characterization elucidates signaling networks that control human ES cell growth and differentiation. *Nat. Biotechnol.* **22**, 707-716.

Branford, W. W. and Yost, H. J. (2002). Lefty-dependent inhibition of nodal- and wnt-responsive organizer gene expression is essential for normal gastrulation. *Curr. Biol.* **12**, 2136-2141.

Branford, W. W. and Yost, H. J. (2004). Nodal signaling: Cryptic Lefty mechanism of antagonism decoded. *Curr. Biol.* **14**, R341-R343.

Brennan, J., Lu, C. C., Norris, D. P., Rodriguez, T. A., Beddington, R. S. and Robertson, E. J. (2001). Nodal signalling in the epiblast patterns the early mouse embryo. *Nature* **411**, 965-969.

Brennan, J., Norris, D. P. and Robertson, E. J. (2002). Nodal activity in the node governs left-right asymmetry. *Genes Dev.* **16**, 2339-2344.

Camus, A., Perea-Gomez, A., Moreau, A. and Collignon, J. (2006). Absence of Nodal signaling promotes precocious neural differentiation in the mouse embryo. *Dev. Biol.* **295**, 743-755.

Chea, H. K., Wright, C. V. and Swalla, B. J. (2005). Nodal signaling and the evolution of deuterostome gastrulation. *Dev. Dyn.* **234**, 269-278.

Chen, C. and Shen, M. M. (2004). Two modes by which Lefty proteins inhibit Nodal signaling. *Curr. Biol.* **14**, 618-624.

Chen, C., Ware, S. M., Sato, A., Houston-Hawkins, D. E., Habas, R., Matzuk, M. M., Shen, M. M. and Brown, C. W. (2006). The Vg1-related protein Gdf3 acts in a Nodal signaling pathway in the pre-gastrulation mouse embryo. *Development* **133**, 319-329.

Chen, S. and Kimelman, D. (2000). The role of the yolk syncytial layer in germ layer patterning in zebrafish. *Development* **127**, 4681-4689.

Chen, Y. and Schier, A. F. (2001). The zebrafish Nodal signal Squint functions as a morphogen. *Nature* **411**, 607-610.

Chen, Y. and Schier, A. F. (2002). Lefty proteins are long-range inhibitors of squint-mediated nodal signaling. *Curr. Biol.* **12**, 2124-2128.

Cheng, S. K., Olale, F., Bennett, J. T., Brivanlou, A. H. and Schier, A. F. (2003). EGF-CFC proteins are essential coreceptors for the TGF-beta signals Vg1 and GDF1. *Genes Dev.* **17**, 31-36.

Cheng, S. K., Olale, F., Brivanlou, A. H. and Schier, A. F. (2004). Lefty blocks a subset of TGFbeta signals by antagonizing EGF-CFC coreceptors. *PLoS Biol.* **2**, 215-226.

Chu, G. C., Dunn, N. R., Anderson, D. C., Oxburgh, L. and Robertson, E. J.

(2004). Differential requirements for Smad4 in TGFbeta-dependent patterning of the early mouse embryo. *Development* **131**, 3501-3512.
Chu, J., Ding, J., Jeays-Ward, K., Price, S. M., Placzek, M. and Shen, M. M. (2005). Non-cell-autonomous role for Cripto in axial midline formation during vertebrate embryogenesis. *Development* **132**, 5539-5551.
Conlon, F. L., Barth, K. S. and Robertson, E. J. (1991). A novel retrovirally induced embryonic lethal mutation in the mouse: assessment of the developmental fate of embryonic stem cells homozygous for the 413.d proviral integration. *Development* **111**, 969-981.
Conlon, F. L., Lyons, K. M., Takaesu, N., Barth, K. S., Kispert, A., Herrmann, B. and Robertson, E. J. (1994). A primary requirement for nodal in the formation and maintenance of the primitive streak in the mouse. *Development* **120**, 1919-1928.
de la Cruz, J. M., Bamford, R. N., Burdine, R. D., Roessler, E., Barkovich, A. J., Donnai, D., Schier, A. F. and Muenke, M. (2002). A loss-of-function mutation in the CFC domain of TDGF1 is associated with human forebrain defects. *Hum. Genet.* **110**, 422-428.
Dickmeis, T., Aanstad, P., Clark, M., Fischer, N., Herwig, R., Mourrain, P., Blader, P., Rosa, F., Lehrach, H. and Strahle, U. (2001). Identification of nodal signaling targets by array analysis of induced complex probes. *Dev. Dyn.* **222**, 571-580.
Ding, J., Yang, L., Yan, Y. T., Chen, A., Desai, N., Wynshaw-Boris, A. and Shen, M. M. (1998). Cripto is required for correct orientation of the anterior-posterior axis in the mouse embryo. *Nature* **395**, 702-707.
Dorey, K. and Hill, C. S. (2006). A novel Cripto-related protein reveals an essential role for EGF-CFCs in Nodal signalling in Xenopus embryos. *Dev. Biol.* **292**, 303-316.
Dougan, S. T., Warga, R. M., Kane, D. A., Schier, A. F. and Talbot, W. S. (2003). The role of the zebrafish nodal-related genes squint and cyclops in patterning of mesendoderm. *Development* **130**, 1837-1851.
Duboc, V. and Lepage, T. (2006). A conserved role for the nodal signaling pathway in the establishment of dorso-ventral and left-right axes in deuterostomes. *J. Exp. Zool. (Mol. Dev. Evol.)* **306B**, 1-13.
Duboc, V., Rottinger, E., Besnardeau, L. and Lepage, T. (2004). Nodal and BMP2/4 signaling organizes the oral-aboral axis of the sea urchin embryo. *Dev. Cell* **6**, 397-410.
Duboc, V., Rottinger, E., Lapraz, F., Besnardeau, L. and Lepage, T. (2005). Left-right asymmetry in the sea urchin embryo is regulated by nodal signaling on the right side. *Dev. Cell* **9**, 147-158.
Dunn, N. R., Vincent, S. D., Oxburgh, L., Robertson, E. J. and Bikoff, E. K. (2004). Combinatorial activities of Smad2 and Smad3 regulate mesoderm formation and patterning in the mouse embryo. *Development* **131**, 1717-1728.
Dunn, N. R., Koonce, C. H., Anderson, D. C., Islam, A., Bikoff, E. K. and Robertson, E. J. (2005). Mice exclusively expressing the short isoform of Smad2 develop normally and are viable and fertile. *Genes Dev.* **19**, 152-163.
Eimon, P. M. and Harland, R. M. (2002). Effects of heterodimerization and proteolytic processing on Derriere and Nodal activity: implications for mesoderm induction in Xenopus. *Development* **129**, 3089-3103.
Feldman, B., Gates, M. A., Egan, E. S., Dougan, S. T., Rennebeck, G., Sirotkin, H. I., Schier, A. F. and Talbot, W. S. (1998). Zebrafish organizer development and germ-layer formation require nodal-related signals. *Nature* **395**, 181-185.
Feldman, B., Concha, M. L., Saude, L., Parsons, M. J., Adams, R. J., Wilson, S. W. and Stemple, D. L. (2002). Lefty antagonism of squint is essential for normal gastrulation. *Curr. Biol.* **12**, 2129-2135.
Flowers, V. L., Courteau, G. R., Poustka, A. J., Weng, W. and Venuti, J. M. (2004). Nodal/activin signaling establishes oral-aboral polarity in the early sea urchin embryo. *Dev. Dyn.* **231**, 727-740.
Germain, S., Howell, M., Esslemont, G. M. and Hill, C. S. (2000). Homeodomain and winged-helix transcription factors recruit activated Smads to distinct promoter elements via a common Smad interaction motif. *Genes Dev.* **14**, 435-451.
Gore, A. V., Maegawa, S., Cheong, A., Gilligan, P. C., Weinberg, E. S. and Sampath, K. (2005). The zebrafish dorsal axis is apparent at the four-cell stage. *Nature* **438**, 1030-1035.
Green, J. B. and Smith, J. C. (1990). Graded changes in dose of a Xenopus activin A homologue elicit stepwise transitions in embryonic cell fate. *Nature* **347**, 391-394.
Grimm, O. H. and Gurdon, J. B. (2002). Nuclear exclusion of Smad2 is a mechanism leading to loss of competence. *Nat. Cell Biol.* **4**, 519-522.
Gripp, K. W., Wotton, D., Edwards, M. C., Roessler, E., Ades, L., Meinecke, P., Richieri-Costa, A., Zackai, E. H., Massague, J., Muenke, M. et al. (2000). Mutations in TGIF cause holoprosencephaly and link NODAL signalling to human neural axis determination. *Nat. Genet.* **25**, 205-208.
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.
Gritsman, K., Talbot, W. S. and Schier, A. F. (2000). Nodal signaling patterns the organizer. *Development* **127**, 921-932.
Gurdon, J. B., Harger, P., Mitchell, A. and Lemaire, P. (1994). Activin signalling and response to a morphogen gradient. *Nature* **371**, 487-492.
Gurdon, J. B., Standley, H., Dyson, S., Butler, K., Langon, T., Ryan, K., Stennard, F., Shimizu, K. and Zorn, A. (1999). Single cells can sense their position in a morphogen gradient. *Development* **126**, 5309-5317.
Haffner, C., Frauli, M., Topp, S., Irmler, M., Hofmann, K., Regula, J. T., Bally-Cuif, L. and Haass, C. (2004). Nicalin and its binding partner Nomo are novel Nodal signaling antagonists. *EMBO J.* **23**, 3041-3050.
Haramoto, Y., Tanegashima, K., Onuma, Y., Takahashi, S., Sekizaki, H. and Asashima, M. (2004). Xenopus tropicalis nodal-related gene 3 regulates BMP signaling: an essential role for the pro-region. *Dev. Biol.* **265**, 155-168.
Harms, P. W. and Chang, C. (2003). Tomoregulin-1 (TMEFF1) inhibits nodal signaling through direct binding to the nodal coreceptor Cripto. *Genes Dev.* **17**, 2624-2629.
Hart, A. H., Hartley, L., Sourris, K., Stadler, E. S., Li, R., Stanley, E. G., Tam, P. P., Elefanty, A. G. and Robb, L. (2002). Mixl1 is required for axial mesendoderm morphogenesis and patterning in the murine embryo. *Development* **129**, 3597-3608.
Hashimoto, H., Rebagliati, M., Ahmad, N., Muraoka, O., Kurokawa, T., Hibi, M. and Suzuki, T. (2004). The Cerberus/Dan-family protein Charon is a negative regulator of Nodal signaling during left-right patterning in zebrafish. *Development* **131**, 1741-1753.
He, W., Dorn, D. C., Erdjument-Bromage, H., Tempst, P., Moore, M. A. and Massague, J. (2006). Hematopoiesis controlled by distinct TIF1gamma and Smad4 branches of the TGFbeta pathway. *Cell* **125**, 929-941.
Hoodless, P. A., Pye, M., Chazaud, C., Labbe, E., Attisano, L., Rossant, J. and Wrana, J. L. (2001). FoxH1 (Fast) functions to specify the anterior primitive streak in the mouse. *Genes Dev.* **15**, 1257-1271.
Iratni, R., Yan, Y. T., Chen, C., Ding, J., Zhang, Y., Price, S. M., Reinberg, D. and Shen, M. M. (2002). Inhibition of excess nodal signaling during mouse gastrulation by the transcriptional corepressor DRAP1. *Science* **298**, 1996-1999.
James, D., Levine, A. J., Besser, D. and Hemmati-Brivanlou, A. (2005). TGFbeta/activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem cells. *Development* **132**, 1273-1282.
Jones, C. M., Kuehn, M. R., Hogan, B. L., Smith, J. C. and Wright, C. V. (1995). Nodal-related signals induce axial mesoderm and dorsalize mesoderm during gastrulation. *Development* **121**, 3651-3662.
Jornvall, H., Reissmann, E., Andersson, O., Mehrkash, M. and Ibanez, C. F. (2004). ALK7, a receptor for nodal, is dispensable for embryogenesis and left-right patterning in the mouse. *Mol. Cell. Biol.* **24**, 9383-9389.
Kato, Y., Habas, R., Katsuyama, Y., Naar, A. M. and He, X. (2002). A component of the ARC/Mediator complex required for TGF beta/Nodal signalling. *Nature* **418**, 641-646.
Kimelman, D. (2006). Mesoderm induction: from caps to chips. *Nat. Rev. Genet.* **7**, 360-372.
Kofron, M., Wylie, C. and Heasman, J. (2004). The role of Mixer in patterning the early Xenopus embryo. *Development* **131**, 2431-2441.
Kunwar, P. S., Zimmerman, S., Bennett, J. T., Chen, Y., Whitman, M. and Schier, A. F. (2003). Mixer/Bon and FoxH1/Sur have overlapping and divergent roles in Nodal signaling and mesendoderm induction. *Development* **130**, 5589-5599.
Le Good, J. A., Joubin, K., Giraldez, A. J., Ben-Haim, N., Beck, S., Chen, Y., Schier, A. F. and Constam, D. B. (2005). Nodal stability determines signaling range. *Curr. Biol.* **15**, 31-36.
Lee, M. A., Heasman, J. and Whitman, M. (2001). Timing of endogenous activin-like signals and regional specification of the Xenopus embryo. *Development* **128**, 2939-2952.
Levin, M., Johnson, R. L., Stern, C. D., Kuehn, M. and Tabin, C. (1995). A molecular pathway determining left-right asymmetry in chick embryogenesis. *Cell* **82**, 803-814.
Levine, A. J. and Brivanlou, A. H. (2006). GDF3, a BMP inhibitor, regulates cell fate in stem cells and early embryos. *Development* **133**, 209-216.
Lewis, S. L. and Tam, P. P. (2006). Definitive endoderm of the mouse embryo: Formation, cell fates, and morphogenetic function. *Dev. Dyn.* **235**, 2315-2329.
Lin, X., Duan, X., Liang, Y. Y., Su, Y., Wrighton, K. H., Long, J., Hu, M., Davis, C. M., Wang, J., Brunicardi, F. C. et al. (2006). PPM1A functions as a Smad phosphatase to terminate TGFbeta signaling. *Cell* **125**, 915-928.
Long, S., Ahmad, N. and Rebagliati, M. (2003). The zebrafish nodal-related gene southpaw is required for visceral and diencephalic left-right asymmetry. *Development* **130**, 2303-2316.
Lowe, L. A., Yamada, S. and Kuehn, M. R. (2001). Genetic dissection of *nodal* function in patterning the mouse embryo. *Development* **128**, 1831-1843.
Marques, S., Borges, A. C., Silva, A. C., Freitas, S., Cordenonsi, M. and Belo, J. A. (2004). The activity of the Nodal antagonist Cerl-2 in the mouse node is required for correct L/R body axis. *Genes Dev.* **18**, 2342-2347.
Massague, J., Seoane, J. and Wotton, D. (2005). Smad transcription factors. *Genes Dev.* **19**, 2783-2810.
Meinhardt, H. and Gierer, A. (2000). Pattern formation by local self-activation and lateral inhibition. *BioEssays* **22**, 753-760.

Meno, C., Shimono, A., Saijoh, Y., Yashiro, K., Mochida, K., Ohishi, S., Noji, S., Kondoh, H. and Hamada, H. (1998). lefty-1 is required for left-right determination as a regulator of lefty-2 and nodal. *Cell* **94**, 287-297.
Meno, C., Gritsman, K., Ohishi, S., Ohfuji, Y., Heckscher, E., Mochida, K., Shimono, A., Kondoh, H., Talbot, W. S., Robertson, E. J. et al. (1999). Mouse Lefty2 and zebrafish antivin are feedback inhibitors of nodal signaling during vertebrate gastrulation. *Mol. Cell* **4**, 287-298.
Meno, C., Takeuchi, J., Sakuma, R., Koshiba-Takeuchi, K., Ohishi, S., Saijoh, Y., Miyazaki, J., ten Dijke, P., Ogura, T. and Hamada, H. (2001). Diffusion of nodal signaling activity in the absence of the feedback inhibitor Lefty2. *Dev. Cell* **1**, 127-138.
Mesnard, D., Guzman-Ayala, M. and Constam, D. B. (2006). Nodal specifies embryonic visceral endoderm and sustains pluripotent cells in the epiblast before overt axial patterning. *Development* **133**, 2497-2505.
Morkel, M., Huelsken, J., Wakamiya, M., Ding, J., van de Wetering, M., Clevers, H., Taketo, M. M., Behringer, R. R., Shen, M. M. and Birchmeier, W. (2003). Beta-catenin regulates Cripto- and Wnt3-dependent gene expression programs in mouse axis and mesoderm formation. *Development* **130**, 6283-6294.
Nakamura, T., Mine, N., Nakaguchi, E., Mochizuki, A., Yamamoto, M., Yashiro, K., Meno, C. and Hamada, H. (2006). Generation of robust left-right asymmetry in the mouse embryo requires a self-enhancement and lateral-inhibition system. *Dev. Cell* **11**, 495-504.
Norris, D. P. and Robertson, E. J. (1999). Asymmetric and node-specific *nodal* expression patterns are controlled by two distinct cis-acting regulatory elements. *Genes Dev.* **13**, 1575-1588.
Norris, D. P., Brennan, J., Bikoff, E. K. and Robertson, E. J. (2002). The FoxH1-dependent autoregulatory enhancer controls the level of Nodal signals in the mouse embryo. *Development* **129**, 3455-3468.
Onuma, Y., Takahashi, S., Haramoto, Y., Tanegashima, K., Yokota, C., Whitman, M. and Asashima, M. (2005). Xnr2 and Xnr5 unprocessed proteins inhibit Wnt signaling upstream of dishevelled. *Dev. Dyn.* **234**, 900-910.
Onuma, Y., Yeo, C. Y. and Whitman, M. (2006). XCR2, one of three Xenopus EGF-CFC genes, has a distinct role in the regulation of left-right patterning. *Development* **133**, 237-250.
Palacios, I. M. and St Johnston, D. (2001). Getting the message across: the intracellular localization of mRNAs in higher eukaryotes. *Annu. Rev. Cell Dev. Biol.* **17**, 569-614.
Perea-Gomez, A., Vella, F. D., Shawlot, W., Oulad-Abdelghani, M., Chazaud, C., Meno, C., Pfister, V., Chen, L., Robertson, E., Hamada, H. et al. (2002). Nodal antagonists in the anterior visceral endoderm prevent the formation of multiple primitive streaks. *Dev. Cell* **3**, 745-756.
Piccolo, S., Agius, E., Leyns, L., Bhattacharyya, S., Grunz, H., Bouwmeester, 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.
Randall, R. A., Howell, M., Page, C. S., Daly, A., Bates, P. A. and Hill, C. S. (2004). Recognition of phosphorylated-Smad2-containing complexes by a novel Smad interaction motif. *Mol. Cell. Biol.* **24**, 1106-1121.
Rankin, C. T., Bunton, T., Lawler, A. M. and Lee, S. J. (2000). Regulation of left-right patterning in mice by growth/differentiation factor-1. *Nat. Genet.* **24**, 262-265.
Raya, A. and Belmonte, J. C. (2006). Left-right asymmetry in the vertebrate embryo: from early information to higher-level integration. *Nat. Rev. Genet.* **7**, 283-293.
Rebagliati, M. R., Toyama, R., Fricke, C., Haffter, P. and Dawid, I. B. (1998a). Zebrafish nodal-related genes are implicated in axial patterning and establishing left-right asymmetry. *Dev. Biol.* **199**, 261-272.
Rebagliati, M. R., Toyama, R., Haffter, P. and Dawid, I. B. (1998b). cyclops encodes a nodal-related factor involved in midline signaling. *Proc. Natl. Acad. Sci. USA* **95**, 9932-9937.
Reissmann, E., Jornvall, H., Blokzijl, A., Andersson, O., Chang, C., Minchiotti, G., Persico, M. G., Ibanez, C. F. and Brivanlou, A. H. (2001). The orphan receptor ALK7 and the Activin receptor ALK4 mediate signaling by Nodal proteins during vertebrate development. *Genes Dev.* **15**, 2010-2022.
Rivera-Perez, J. A., Mager, J. and Magnuson, T. (2003). Dynamic morphogenetic events characterize the mouse visceral endoderm. *Dev. Biol.* **261**, 470-487.
Robertson, E., Bradley, A., Kuehn, M. and Evans, M. (1986). Germ-line transmission of genes introduced into cultured pluripotential cells by retroviral vector. *Nature* **323**, 445-448.
Rodriguez, T. A., Srinivas, S., Clements, M. P., Smith, J. C. and Beddington, R. S. (2005). Induction and migration of the anterior visceral endoderm is regulated by the extra-embryonic ectoderm. *Development* **132**, 2513-2520.
Ross, S., Cheung, E., Petrakis, T. G., Howell, M., Kraus, W. L. and Hill, C. S. (2006). Smads orchestrate specific histone modifications and chromatin remodeling to activate transcription. *EMBO J.* **25**, 4490-4502.
Rossant, J. and Tam, P. P. (2004). Emerging asymmetry and embryonic patterning in early mouse development. *Dev. Cell* **7**, 155-164.
Saijoh, Y., Adachi, H., Sakuma, R., Yeo, C. Y., Yashiro, K., Watanabe, M., Hashiguchi, H., Mochida, K., Ohishi, S., Kawabata, M. et al. (2000). Left-right asymmetric expression of lefty2 and nodal is induced by a signaling pathway that includes the transcription factor FAST2. *Mol. Cell* **5**, 35-47.
Saijoh, Y., Oki, S., Ohishi, S. and Hamada, H. (2003). Left-right patterning of the mouse lateral plate requires nodal produced in the node. *Dev. Biol.* **256**, 161-173.
Saijoh, Y., Oki, S., Tanaka, C., Nakamura, T., Adachi, H., Yan, Y. T., Shen, M. M. and Hamada, H. (2005). Two nodal-responsive enhancers control left-right asymmetric expression of Nodal. *Dev. Dyn.* **232**, 1031-1036.
Sakuma, R., Ohnishi Yi, Y., Meno, C., Fujii, H., Juan, H., Takeuchi, J., Ogura, T., Li, E., Miyazono, K. and Hamada, H. (2002). Inhibition of Nodal signalling by Lefty mediated through interaction with common receptors and efficient diffusion. *Genes Cells* **7**, 401-412.
Sampath, K., Rubinstein, A. L., Cheng, A. M., Liang, J. O., Fekany, K., Solnica-Krezel, L., Korzh, V., Halpern, M. E. and Wright, C. V. (1998). Induction of the zebrafish ventral brain and floorplate requires cyclops/nodal signalling. *Nature* **395**, 185-189.
Schier, A. F. (2003). Nodal signaling in vertebrate development. *Annu. Rev. Cell Dev. Biol.* **19**, 589-621.
Schier, A. F. and Shen, M. M. (2000). Nodal signalling in vertebrate development. *Nature* **403**, 385-389.
Schier, A. F. and Talbot, W. S. (2005). Molecular genetics of axis formation in zebrafish. *Annu. Rev. Genet.* **39**, 561-613.
Schier, A. F., Neuhauss, S. C. F., Helde, K. A., Talbot, W. S. and Driever, W. (1997). The *one-eyed pinhead* gene functions in mesoderm and endoderm formation in zebrafish and interacts with *no tail*. *Development* **124**, 327-342.
Shen, M. M. and Schier, A. F. (2000). The *EGF-CFC* gene family in vertebrate development. *Trends Genet.* **16**, 303-309.
Shimizu, K. and Gurdon, J. B. (1999). A quantitative analysis of signal transduction from activin receptor to nucleus and its relevance to morphogen gradient interpretation. *Proc. Natl. Acad. Sci. USA* **96**, 6791-6796.
Shiratori, H. and Hamada, H. (2006). The left-right axis in the mouse: from origin to morphology. *Development* **133**, 2095-2104.
Silva, A. C., Filipe, M., Kuerner, K. M., Steinbeisser, H. and Belo, J. A. (2003). Endogenous Cerberus activity is required for anterior head specification in Xenopus. *Development* **130**, 4943-4953.
Sirotkin, H. I., Gates, M. A., Kelly, P. D., Schier, A. F. and Talbot, W. S. (2000). *Fast1* is required for the development of dorsal axial structures in zebrafish. *Curr. Biol.* **10**, 1051-1054.
Skromne, I. and Stern, C. D. (2001). Interactions between Wnt and Vg1 signalling pathways initiate primitive streak formation in the chick embryo. *Development* **128**, 2915-2927.
Song, J., Oh, S. P., Schrewe, H., Nomura, M., Lei, H., Okano, M., 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.
Srinivas, S., Rodriguez, T., Clements, M., Smith, J. C. and Beddington, R. S. (2004). Active cell migration drives the unilateral movements of the anterior visceral endoderm. *Development* **131**, 1157-1164.
Stainier, D. Y. (2002). A glimpse into the molecular entrails of endoderm formation. *Genes Dev.* **16**, 893-907.
Strahle, U., Jesuthasan, S., Blader, P., Garcia-Villalba, P., Hatta, K. and Ingham, P. W. (1997). one-eyed pinhead is required for development of the ventral midline of the zebrafish (Danio rerio) neural tube. *Genes Funct.* **1**, 131-148.
Strizzi, L., Bianco, C., Normanno, N., Seno, M., Wechselberger, C., Wallace-Jones, B., Khan, N. I., Hirota, M., Sun, Y., Sanicola, M. et al. (2004). Epithelial mesenchymal transition is a characteristic of hyperplasias and tumors in mammary gland from MMTV-Cripto-1 transgenic mice. *J. Cell. Physiol.* **201**, 266-276.
Strizzi, L., Bianco, C., Normanno, N. and Salomon, D. (2005). Cripto-1: a multifunctional modulator during embryogenesis and oncogenesis. *Oncogene* **24**, 5731-5741.
Sun, Y., Strizzi, L., Raafat, A., Hirota, M., Bianco, C., Feigenbaum, L., Kenney, N., Wechselberger, C., Callahan, R. and Salomon, D. S. (2005). Overexpression of human Cripto-1 in transgenic mice delays mammary gland development and differentiation and induces mammary tumorigenesis. *Am. J. Pathol.* **167**, 585-597.
Takaoka, K., Yamamoto, M., Shiratori, H., Meno, C., Rossant, J., Saijoh, Y. and Hamada, H. (2006). The mouse embryo autonomously acquires anterior-posterior polarity at implantation. *Dev. Cell* **10**, 451-459.
Tao, Q., Yokota, C., Puck, H., Kofron, M., Birsoy, B., Yan, D., Asashima, M., Wylie, C. C., Lin, X. and Heasman, J. (2005). Maternal Wnt11 activates the canonical Wnt signaling pathway required for axis formation in Xenopus embryos. *Cell* **120**, 857-871.
Thisse, B., Wright, C. V. and Thisse, C. (2000). Activin- and Nodal-related factors

control antero-posterior patterning of the zebrafish embryo. *Nature* **403**, 425-428.
Thomas, P. Q., Brown, A. and Beddington, R. S. P. (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.
Topczewska, J. M., Postovit, L. M., Margaryan, N. V., Sam, A., Hess, A. R., Wheaton, W. W., Nickoloff, B. J., Topczewski, J. and Hendrix, M. J. (2006). Embryonic and tumorigenic pathways converge via Nodal signaling: role in melanoma aggressiveness. *Nat. Med.* **12**, 925-932.
Vallier, L., Reynolds, D. and Pedersen, R. A. (2004). Nodal inhibits differentiation of human embryonic stem cells along the neuroectodermal default pathway. *Dev. Biol.* **275**, 403-421.
Vallier, L., Alexander, M. and Pedersen, R. A. (2005). Activin/Nodal and FGF pathways cooperate to maintain pluripotency of human embryonic stem cells. *J. Cell Sci.* **118**, 4495-4509.
Vincent, S. D., Dunn, N. R., Hayashi, S., Norris, D. P. and Robertson, E. J. (2003). Cell fate decisions within the mouse organizer are governed by graded Nodal signals. *Genes Dev.* **17**, 1646-1662.
Vincent, S. D., Norris, D. P., Le Good, J. A., Constam, D. B. and Robertson, E. J. (2004). Asymmetric Nodal expression in the mouse is governed by the combinatorial activities of two distinct regulatory elements. *Mech. Dev.* **121**, 1403-1415.
Wall, N. A., Craig, E. J., Labosky, P. A. and Kessler, D. S. (2000). Mesendoderm induction and reversal of left-right pattern by mouse Gdf1, a Vg1-related gene. *Dev. Biol.* **227**, 495-509.
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.
Whitman, M. (2001). Nodal signaling in early vertebrate embryos. Themes and variations. *Dev. Cell* **1**, 605-617.
Williams, P. H., Hagemann, A., Gonzalez-Gaitan, M. and Smith, J. C. (2004). Visualizing long-range movement of the morphogen Xnr2 in the Xenopus embryo. *Curr. Biol.* **14**, 1916-1923.
Yamamoto, M., Meno, C., Sakai, Y., Shiratori, H., Mochida, K., Ikawa, Y., Saijoh, Y. and Hamada, H. (2001). The transcription factor FoxH1 (FAST) mediates Nodal signaling during anterior-posterior patterning and node formation in the mouse. *Genes Dev.* **15**, 1242-1256.
Yamamoto, M., Mine, N., Mochida, K., Sakai, Y., Saijoh, Y., Meno, C. and Hamada, H. (2003). Nodal signaling induces the midline barrier by activating Nodal expression in the lateral plate. *Development* **130**, 1795-1804.
Yamamoto, M., Saijoh, Y., Perea-Gomez, A., Shawlot, W., Behringer, R. R., Ang, S. L., Hamada, H. and Meno, C. (2004). Nodal antagonists regulate formation of the anteroposterior axis of the mouse embryo. *Nature* **428**, 387-392.
Yan, Y.-T., Gritsman, K., Ding, J., Burdine, R. D., Corrales, J. D., Price, S. M., Talbot, W. S., Schier, A. F. and Shen, M. M. (1999). Conserved requirement for *EGF-CFC* genes in vertebrate left-right axis formation. *Genes Dev.* **13**, 2527-2537.
Yan, Y.-T., Liu, J.-J., Luo, Y., Chaosu, E., Haltiwanger, R. S., Abate-Shen, C. and Shen, M. M. (2002). Dual roles of Cripto as a ligand and coreceptor in the Nodal signaling pathway. *Mol. Cell. Biol.* **22**, 4439-4449.
Yeo, C. and Whitman, M. (2001). Nodal signals to Smads through Cripto-dependent and Cripto-independent mechanisms. *Mol. Cell* **7**, 949-957.
Zhang, J., Talbot, W. S. and Schier, A. F. (1998). Positional cloning identifies zebrafish *one-eyed pinhead* as a permissive EGF-related ligand required during gastrulation. *Cell* **92**, 241-251.
Zhang, L., Zhou, H., Su, Y., Sun, Z., Zhang, H., Zhang, L., Zhang, Y., Ning, Y., Chen, Y. G. and Meng, A. (2004). Zebrafish Dpr2 inhibits mesoderm induction by promoting degradation of nodal receptors. *Science* **306**, 114-117.
Zhou, X., Sasaki, H., Lowe, L., Hogan, B. L. and Kuehn, M. R. (1993). Nodal is a novel TGF-beta-like gene expressed in the mouse node during gastrulation. *Nature* **361**, 543-547.