Establishing a Left-Right Axis in the Embryo

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Summary

Vertebrates exhibit evolutionarily conserved asymmetries in the pattern of internal organ placement that are essential for their normal physiological function. Left-right asymmetries in organ situs are dependent upon the formation of an intact left-right axis during embryogenesis. Recently many of the molecular components involved in the initiation and maintenance of the left-right axis have been described. These molecules and their function in promoting left-right asymmetries are reviewed.

Keywords  Asymmetry; left-right axis; organ situs.

INTRODUCTION

A fundamental aspect of vertebrate embryogenesis are the events responsible for patterning the embryo as it develops from a single-celled fertilized egg into a complex multicellular organism. Understanding the mechanisms responsible for distinguishing the left and right sides of the embryo relative to its anterior-posterior and dorsal-ventral axes has been a main focus of recent research. Although vertebrates are essentially bilaterally symmetrical with respect to their external features, very dramatic internal asymmetries develop around their midline axis during embryogenesis. For instance, the heart, stomach, and spleen are invariably on the left, whereas nearly all of the liver is on the right. The positioning of the internal organs with respect to the midline is highly conserved between species and is referred to as situs solitus. Alterations in this classic pattern may occur as a complete mirror image reversal of all of the organs (situs inversus), reversal of individual organs along the left-right axis (heterotaxia), or changes in normal symmetry or aberrant bilateral symmetry of a particular organ (isomerism) (Figure 1). Except for complete situs inversus, the physiological consequence of laterality defects in internal organ positioning is usually severe (1, 2). The series of embryonic developmental events that distinguish the left and right sides of the embryo along its anterior-posterior and dorsal-ventral axes include the direction of axial rotation, morphogenesis of individual organs, and organ placement. The findings derived from research aimed at characterizing the molecules responsible for patterning the left-right axis of the embryo form the basis of this review.

FORMATION OF THE LEFT–RIGHT AXIS

The initial phases of embryogenesis are extremely variable among vertebrates, particularly with respect to the process of gastrulation and establishment of the three germ layers. Nonetheless, the hierarchy of signaling molecules responsible for determining the initial anterior-posterior and dorsal-ventral axes of the embryo appears to be conserved (3). In chicks, a somewhat labile anterior-posterior axis develops between 20 and 22 h postfertilization (4), and the dorsal-ventral axis develops shortly thereafter. In mice, the nascent anterior-posterior and dorsal-ventral axes are not defined until formation of the primitive streak ~6.5 days postcoitus (5). In both species, the left-right axis is physically defined as a consequence of the establishment of the anterior-posterior and dorsal-ventral axes. Formation of the left-right axis has been examined in several model systems. Studies in chick and mouse embryos have led to the identification of many of the molecular components that pattern the left and right sides of the embryo and have permitted a comparison of the mechanisms responsible for initiating left-right axis development.

The earliest conserved morphological disruption of bilateral symmetry observed in all vertebrate embryos is the rightward
looping of the heart, which is determined during gastrulation (5, 6). This is followed by rotation of the embryo along its anterior–posterior axis and twisting along the rostral caudal axis in a counter-clockwise rotation. Coinciding with axial rotation, the most lateral portions of the body wall grow toward each other and fuse along the ventral midline. Even closure of the ventral body wall exhibits left-right differences, in that the mesoderm on the lateral edges of the embryo proliferates more on the left side than on the right (7). Additionally, each individual organ is morphologically patterned around three axes, and bilaterally paired organs, such as the lung, exhibit differences in the number of lobes on the left and right sides of the body. Development of the characteristic pattern of organ situs is coincident with the process of embryonic turning.

Developmental cues that direct all aspects of left-right patterning occur well in advance of any visible sign of morphological asymmetry. As is true for the anterior–posterior and dorsal–ventral axes, spatially asymmetric patterns of gene expression precede the appearance of a morphologically observable left–right axis. Indeed, roles for several of the molecules that exhibit left–right asymmetries in their expression patterns have been demonstrated through gain-of-function and loss-of-function experiments in vivo. In addition, alterations in the expression patterns of many of these factors are observed in embryos with situs defects. These molecules predominantly fall into two categories: signaling molecules and their receptors, and transcription factors that integrate the information from various signaling pathways to regulate the expression of downstream target genes. Structural proteins have also been shown to be critical components of the left–right patterning cascade. Molecular components and their relative positions in the left–right patterning cascade are diagrammatically represented in Figure 2.
Setting the Cascade in Motion: Establishing Left–Right Differences at the Node

During the past decade several hypotheses regarding initiation of left–right patterning have been proposed (8, 9). Although the trigger that sets the cascade in motion remains elusive, a structurally and molecularly intact node clearly is essential. The mouse node, which is the functional equivalent of Hensen’s node in the chick and Spemann’s organizer in Xenopus, has a unique, bilaminar architecture, in which each cell in the ventral layer has a single motile cilium (10). These cilia project into the extraembryonic fluid, where their coordinated vortical movement generates a leftward current across the node, referred to as “nodal flow” (11–13). Several observations support the hypothesis that the presence and motility of these cilia are important for correct patterning along the left–right axis of the embryo. In humans, syndromic laterality defects are most often observed as one of the manifestations of immotile cilia syndrome (14). Analysis of laterality defects in several mutant mouse strains, including the inversus viscerum mice (iv [15]) and the inversion of embryo turning mice (inv [16]), supports a role for nodal flow. The inv/inv mice exhibit randomized laterality as a result of a point mutation in the gene encoding left–right dynein (Lrd), an axonemal dynein heavy-chain molecule (17, 18), whereas inv/inv mice exhibit complete situs inversus resulting from a transgene insertion into the inversin locus (19, 20). The cilia in inv/inv mice are immotile; those in inv/inv animals generate only weak nodal flow (13). Recently, mice with mutations in the loci encoding the KIF3A (12, 21) and KIF3B (11) components of molecular motors, and in the forkhead transcription factor Hnf-4/HFH-4 (22), were shown to lack nodal cilia and exhibit laterality defects. Although nodal flow is presumed to promote the asymmetric distribution of molecules in the vicinity of the node, the precise nature of this factor or factors is not known. A requirement for a structurally intact node in left–right patterning is further supported by node ablation studies (23).

Cilia have not been observed on the cells that make up Hensen’s node in the chick or the blastopore lip in Xenopus. In the chick, gap junction communication between the left and right halves of the blastoderm-stage embryo (24, 25), and asymmetric gene expression in the node, represent the earliest identified events of left–right patterning. Activin receptor IIA (ActRIIA) and Sonic hedgehog (Shh) were the first genes reported to be expressed asymmetrically in the vicinity of Hensen’s node (26–28). Shh is initially expressed throughout the node, but coincident with the onset of ActRIIA expression on the right, Shh expression is restricted to the left side. Application of ectopic activin on the left side of the node induces the expression of ActRIIA and represses Shh, suggesting that activin, or an activin-like activity, is near the beginning of the signaling cascade, where at least one of its functions is to restrict Shh expression to the left side of the node. Misexpression of Shh on the right side of the embryo causes randomization of both heart looping and visceral situs (28), presumably because of ectopic activation of several genes that ordinarily are expressed only on the left side of the embryo, including Nodal and Pitx2 (29–33). The presence of antibodies that block SHH activity inhibits the expression of downstream target genes on the left side of the embryo. Thus, SHH appears to be important for promoting “leftness” in the chick.

Recently, asymmetric expression of Fgf8 in the posterior portion of the right side of Hensen’s node was observed in the chick (34). The right-sided expression of Fgf8 is maintained throughout the four-somite stage, is spatially and temporally distinct from that of Shh and cActRIAIA, and appears to be downstream of activin signaling, upstream of the gene encoding the zinc-finger protein Snail-Related (cSnR), and independent of SHH (34). Misexpression of Fgf8 on the left side of the embryo represses Nodal and Pitx2, which ordinarily are expressed in the left lateral plate mesoderm, and, ultimately, can affect heart looping and cause isomerisms. Thus, in the chick the FGF8 signaling pathway appears to be a critical regulator of the events that affect “rightness” by promoting the expression of right-sided genes and inhibiting the expression of left-sided genes.

Shh, ActRIIA, and Fgf8 are not asymmetrically expressed in mice, and laterality defects were not observed in mice with targeted gene deletions of activin βA or βB (35), or ActRIIA (36) loci, or in the initial characterization of the Shh null mouse (37). These data suggest that the nature of the initial molecular events required to establish left versus right might not be evolutionarily conserved. However, situs defects were observed in the ActRIIB knock-out mice (38), in further analysis of the Shh knock-out mice (39–42), and in embryos that were compound heterozygotes for mutations at the Fgf8 genomic locus (42), indicating a potentially conserved role for these molecules in patterning the left–right axis. At first glance, each seems to pattern the opposite side of the murine left–right axis from what would have been predicted, based on their observed asymmetric expression patterns and the consequences of their misexpressions in the chick. For instance, right pulmonary isomerisms were observed in the ActRIIB null (38) and Fgf8 compound heterozygotes (42), and left pulmonary isomerisms were found in Shh null embryos (39–42). Shh null mice also exhibited random turning of the body axis, and although cardiac looping was normal, it was often delayed or incomplete, such that the heart was abnormally positioned relative to the midline. In addition, several genes ordinarily expressed in the left lateral plate mesoderm (Nodal, Lefty-2, and Pitx2) and thought to be downstream of SHH signaling in the chick were observed to be ectopically induced in the anterior portion of the right lateral plate mesoderm in Shh null mice. In contrast, the absence of Lefty-1 expression on the left side of the floorplate in Shh /−/− embryos was in agreement with the observation that an SHH-blocking antibody represses Lefty-1 expression in the chick. Thus, perhaps the ectopic gene expression observed in the right lateral plate mesoderm of Shh null mice is a consequence of disruption of Lefty-1 expression. (See discussion of Lefty-1 gene deletion below.)

The role of FGF8 in patterning the left–right axis also seems to be reversed in the mouse embryo versus the chick. Abnormal heart looping and right pulmonary isomerisms, individually or combined, were observed in ~50% of the embryos that were compound heterozygotes for mutations at the Fgf8 locus (42). Right pulmonary isomerisms were sometimes observed in
Figure 2. Asymmetric gene expression on the left and right sides of the embryo. Schematic representation of the asymmetric patterns of gene expression that have been described in chick (a) and mouse (b) embryos are shown in the upper portion of each panel. In these ventral views of the embryo, the left side of the embryo is on the reader’s right. (a) In the early chick node (stages 4 through 6), Shh (dark green) is expressed on the left side of the node (illustrated by a circle). An activin-like signal on the right side of the node (red), probably Activin βB, restricts Shh expression to the left and causes the upregulation of ActRIIA and Fgf8 in the right half of the node. Shh expression is upstream of lefty-1 expression (light green) in the left node and left side of the midline and is upstream of Nodal expression (blue) in the cells immediately to the left of the node at stage 5 (5).
combination with abnormal situs of the visceral organs or right isomerisms of the heart. Complete situs inversus was observed in 4% of these embryos. The effect on gene expression in the lateral plate mesoderm was completely the opposite of what would have been predicted from studies in the chick. The normal left-sided expression of Nodal, Lefty-2, and Pitx2, was absent in ~50% of the compound heterozygotes. Thus, these data support a role for Shh and FGF8 in patterning the left–right axis in both model systems. However, in contrast to the chick, FGF8 appears to be the critical factor for “leftness” in the mouse.

Retinoic acid (RA), the active derivative of vitamin A, is an extremely powerful morphogen, having multiple roles during embryogenesis (43, 44). Both excesses and deficiencies in RA may induce laterality defects (40, 45–49). Retinaldehyde dehydrogenase 2, the enzyme primarily responsible for catalyzing the conversion of vitamin A to retinoic acid, is excluded from the node but is expressed bilaterally in the surrounding mesenchyme. Treating embryos with a retinoic acid antagonist downregulates the expression of genes that ordinarily are expressed in the left lateral plate mesoderm, including Nodal and Pitx2, whereas using high doses of retinoic acid itself results in ectopic expression of these same genes in the right lateral plate.

Abbreviation: BMP, bone morphogenetic; RA, retinoic acid; TGFβ, transforming growth factor-β.

Subsequently at stage 7, Nodal is expressed in a domain of the left lateral plate mesoderm (blue 7). Caronte initially is expressed bilaterally in the anterior region of the early chick embryo (stage 4, not shown), before becoming restricted to a domain in the left lateral plate mesoderm at stage 7 (brown). Also at stage 7, the Bmps are bilaterally expressed in the lateral plate mesoderm (red stippling). Expression of SnR (yellow) is induced by FGF8 on the right and inhibited by Nodal on the left. Expression of Pitx2 (aqua) in the left lateral plate mesoderm is downstream of Nodal signaling. (b) In the mouse, the monociliated cells of the node (ζ) have been shown to establish a net leftward movement of molecules across the node, which is known as “nodal flow” and indicated by the arrow. Not represented in this diagram is the symmetrical expression of Fgf8 in the primitive streak or the expression of Shh in the axial mesendoderm (future notochord) between E7.5 and 8.5 of mouse embryogenesis. Because Shh, Fgf8, ActivinβB, and ActRIIA are symmetrically expressed in the mouse node, the leftward nodal flow is believed to be responsible for the asymmetric expression of Nodal in the node and in the cells adjacent to the left side of the node (blue). As in the chick, Lefty-1 is expressed asymmetrically in the left side of the midline (light green), and BMPs are expressed bilaterally on the left and right sides of the embryo (red stippling). At a slightly later stage (E8), Nodal is asymmetrically expressed in the left lateral plate mesoderm. Subsequently, Lefty-2 and Pitx2 are asymmetrically expressed in a broader domain in the left lateral plate mesoderm (aqua). The lower portion of each panel illustrates the proposed models for the molecular/biochemical relationship of these molecules with respect to their roles in establishing the left–right axis of the embryo. In the chick (a, lower), an activin-like signal, presumably ActivinβB, induces the expression of the activin receptor (ActRIIA) and Fgf-8. Together, these signals act to restrict Shh to the left side of the node. Nodal expression in the cells adjacent to the node is induced by SHH. Subsequently, Caronte antagonizes the repression of Nodal by the BMPs; Nodal is also expressed in the left lateral plate mesoderm, where it induces expression of Pitx2 and inhibits expression of SnR. SnR represses expression of Pitx2 on the right. In the mouse (b, lower), leftward nodal flow is believed to compensate for the apparent absence of asymmetric gene expression in the early mouse node in establishing left versus right differences in gene expression. Listed are some of the molecules that have been demonstrated genetically to be important for the motility of the nodal cilia, for the integrity of the node or midline (or both) and thus for the initial establishment of the left–right axis. The specific functions of these molecules are described in detail in the text. A mammalian homolog of Caronte has not yet been identified, however, a Caronte-like activity is presumed to antagonize the repression of Nodal expression in the left lateral plate mesoderm by BMPs. In mouse, BMP repression of Nodal appears to be mediated by Smad-5. Nodal is then capable of inducing expression of Lefty-2 and Pitx2 and autoregulating its own expression, probably through ActRIIB and Smad-2. A role for retinoic acid (RA) in establishing the left–right axis has demonstrated at multiple points, as indicated by the arrows. Whether these effects are direct or indirect, however, is unknown.

Maintaining the Signal: Balancing Activities of the TGFβ Superfamily

One of the unique features of patterning the left-right axis of the embryo is that, once established, gradients of information that distinguish the left and right sides of the embryo need to be maintained and prevented from crossing the midline. Analysis of the notail and floatinghead mutations in zebrafish (50) and the No turning (51) and Hnf3β mutant mice (52–54), all of which lack midline structures, in conjunction with extirpation of the notochord in Xenopus (55), led to the proposal that the midline functions as a barrier to prevent the spread of information encoding leftness to the right and information encoding rightness to the left. Analysis of the Sil and lefty-1 mutations, which cause laterality defects in the absence of apparent structural alterations in the midline (41, 56), suggests that the midline acts as a barrier at the molecular level as well as at a structural level. At the molecular level, several components of the transforming growth factor-β (TGFβ) signaling cascade appear to fulfill the role of maintaining and propagating the left-right asymmetries initiated by SHH and FGF8 and are at least partially responsible...
for restricting left and right information to the appropriate sides of the embryo. The integrated function of at least three classes of TGFβ signaling molecules are required during this phase of left-right patterning: Nodal, which is expressed not only in the node, as its name implies, but also along the entire anterior-posterior axis of the left lateral plate mesoderm and in a few cells of the left floor plate (53, 57); Lefty-1 and Lefty-2/antivin, which are expressed in the presumptive left floorplate and in the left lateral plate mesoderm, respectively (58–61); and, finally, the bone morphogenetic proteins (BMPs) 2, 4, and 7, which are expressed bilaterally in the lateral plate mesoderm of stage 7–8 chick embryos and along the primitive streak (62, 63).

Experiments in several species have demonstrated a critical role for Nodal and Lefty in patterning the left-right axis (64). Their expression in the left lateral plate mesoderm is conserved among all vertebrate species examined to date. In the chick, the expression of Nodal and lefty appears to be induced by SHH on the left side and inhibited by FGF8 on the right. In mice, the expression of Nodal and lefty-2 in the left lateral plate mesoderm appears to be regulated by similar enhancer elements (65–67), and their expression patterns are similarly disrupted in the iv and inv mice (53, 57–59) and in mice harboring targeted deletions/mutations in the Shh (39–42), Fgf8 (42), and KIF loci (11, 12, 21). Deletion of the murine Shh locus results in bilateral expression of Nodal and lefty-2 in the lateral plate mesoderm, whereas expression of both genes is absent in mice harboring compound mutations at the Fgf8 locus. Ectopic misexpression of either Nodal or lefty-2 on the right-handside of the chick embryo, or inhibition of their expression on the left, randomizes heart-looping and induces laterality defects (68, 69), and both genes are capable of inducing expression of the downstream homeodomain transcription factor, Pitx2 (29–31, 33, 70). The absence of either Nodal (71) or lefty-2 (72) results in early embryonic lethality, precluding independent characterization of their effects on the left–right axis.

Targeted mutagenesis of the lefty-1 locus resulted in various left–right positional defects, confirming its role in the left-right asymmetry cascade (56). The most common laterality defect in lefty-1 mutants was left pulmonary isomerism, as was observed in the Shh mutants. In the absence of lefty-1, Nodal and lefty-2 were initially induced only on the left side of the embryo; subsequently, they were expressed bilaterally in the lateral plate mesoderm, as was Pitx2. These data suggest that Lefty-1 is an important molecular component of the midline barrier. Interestingly, in the chick, excess Lefty-1 on the left side blocks activation of Nodal by Caronte (see below and 62, 63), and ectopic expression of lefty-1 on the right side downregulates expression of Fgf8 and cSnr and upregulates Caronte, Nodal, and Pitx2 (62).

Lefty-1 and Lefty-2 are missing both the α-helix that is involved in dimerization of TGFβ family members and the conserved cysteine residue that ordinarily stabilizes the dimer (58–60). This observation has led to the proposal that Lefty may function as an extracellular antagonist of Nodal signaling. Analysis of the combined Nodal and lefty-2 mutations in mice and zebrafish has provided further evidence in support of their potential antagonistic activity (60, 61, 72). lefty-2 null mice have a lateral expansion of the primitive streak, which results in the formation of excess mesoderm (72). In contrast, Nodal null mice are impaired in primitive streak formation and consequently largely devoid of mesoderm (71). Nodal expression is upregulated and laterally expanded in lefty-2 null mice, whereas lefty-2 transcripts are absent in Nodal mutant mice (72). Crossing the Nodal mutation with lefty-2 knock-out mice partially rescues the lefty-2 null phenotype, suggesting that Nodal and Lefty-2 have antagonistic genetic interactions (72). A similar genetic relationship for these molecules has been described in zebrafish (60, 61). In the absence of Nodal signaling, Lefty/antivin expression is initiated but not maintained at the dorsal margin, and the lateral and ventral expression domains are completely dependent on Nodal signaling. Overexpression of Nodal leads to widespread overexpression of antivin and presumably induces a negative feedback loop. These data suggest that one of the roles of Lefty-2 during gastrulation is to attenuate Nodal signaling, perhaps by blocking Nodal’s ability to interact with its receptor. This mechanism of regulation between Nodal and Lefty during mesoderm induction can probably be extended to how these molecules function to regulate the activity of one another during establishment of the left–right axis. Intriguingly, misexpression of either the agonist Nodal or its presumed antagonist, Lefty-2, is capable of ectopically inducing Pitx2 expression in the lateral plate mesoderm.

The inability of SHH to induce Nodal in lateral plate mesoderm explant cultures in the absence of paraxial mesoderm suggests that the tissue immediately lateral to the node may be important for transferring the information from the node to the lateral plate mesoderm (73). The expression of Patched, a cell-surface molecule believed to be the receptor for SHH and that participates in a negative feedback loop with SHH in the perinodal region, suggests that SHH may function as a short-range signaling molecule in this pathway (73). Two new players in the TGFβ signaling cascade have been demonstrated to function in the relay of information from the node to the lateral plate mesoderm. These are the epidermal growth factor–CFC family members cryptic/one-eyed pinhead and the Cerberus-related molecule Caronte.

Cryptic is expressed symmetrically in the lateral plate mesoderm prior to the asymmetric expression of Nodal and lefty-2 (74), and its presence is required for cells to respond to Nodal (75). Cryptic null mice exhibit a wide spectrum of laterality defects, including right pulmonary isomerism and asplenia (76). Heart looping and embryonic turning are randomized in these mutant embryos, and lefty-1, lefty-2, and Pitx2 are not expressed in the left lateral plate mesoderm. Although Nodal expression is observed in the perinodal region, it is never turned on in the lateral plate mesoderm. Mutations in one-eyed pinhead, the zebrafish homolog of cryptic, have similar effects on laterality, and marker genes that ordinarily are expressed asymmetrically in the lateral plate mesoderm are not expressed (76). These findings suggest that cryptic is required in the lateral plate mesoderm to mediate the response to the “left-side” signal that emanates from the node.
On the basis of several observations, Caronte has been proposed to function as the missing factor that links the SHH signal at the node to asymmetric Nodal expression in the left lateral plate mesoderm (62, 63, 77). Caronte, initially expressed bilaterally in the perinodal region of the stage 4 embryo, is down-regulated on the right such that by stage 7 it is expressed in a restricted zone in the left paraxial mesoderm, adjacent to the Shh-expressing cells of the node. Caronte then is expressed in the left lateral plate mesoderm until stage 10. In chick, Caronte is induced by SHH and repressed by FGF8. Ectopic Caronte on the right side of the embryo induces Nodal and randomizes heart looping. As a member of the Cerberus/Dan family, Caronte is capable of interacting specifically with certain members of the TGFβ superfamily, including Nodal and BMPs, to abrogate their signaling activity. The interaction of Caronte with the bilaterally expressed BMPs on the left side of the embryo is believed to relieve the inhibitory effect of BMPs on Nodal expression in the left lateral plate mesoderm. Transplanted Caronte-producing cells adjacent to the right notochord induce lefty-1 expression on the right, suggesting that Caronte antagonism of BMP is also required to induce lefty-1. Overexpression of BMPs on the left side of the embryo antagonizes the expression of Nodal, suggesting that a balance between Caronte and BMP expression is critical.

A role for two antagonistic TGFβ-related signaling pathways that function on opposite sides of the embryo to generate left-right asymmetries has also been proposed in Xenopus. Gap junction communication and Vg1, a TGFβ-related signaling molecule, are genetically upstream of Nodal expression in the left lateral plate mesoderm (78, 79). Misexpression of mature Vg1 on the right elicits left-right reversals, as does expression of a dominant negative receptor that blocks Vg1 activity on the left (78, 79). Although ectopic BMP receptor ligands can reverse the direction of heart looping (79), misexpression of BMP in conjunction with Vg1 attenuates their individual abilities to alter heart looping. A requirement for right-sided expression of the activin-like kinase receptor (ALK2) upstream of Nodal has been demonstrated (80). These data support a model wherein an active signaling cascade on the right side of the embryo would act antagonistically to the left-side signaling cascade to establish the left-right axis in the Xenopus blastula.

In all species, the temporal and spatial activity of the TGFβ-signaling component of the left-right patterning cascade appears to be precisely regulated through a balance of agonists, antagonists, and extracellular factors. Subtle changes in the expression of any of these components could, therefore, affect the range and duration of Nodal signaling. This mode of regulation may permit individual organ primordia along the anterior–posterior axis of the embryo to respond independently to local changes in the amount of Nodal signaling activity.

**Converting the Signal into Morphological Asymmetry: Role of Transcription Factors**

Transcription factors are responsible for converting the information provided by signaling molecules at various points in the left-right signaling cascade into changes in gene expression that will ultimately generate the morphological asymmetries that define the left-right axis. These transcription factors fall into two categories: transcription factors that are directly activated as a consequence of ligand–receptor interaction, and those that are expressed in response to a signal. The ubiquitously expressed Smad family of transcription factors falls into the first category (81). Smad-1, -2, -3, and -5 are cytoplasmic transcription factors that interact with type I receptors for TGFβ ligands. When the ligands interacts with the receptor, the Smad protein is phosphorylated and released from the receptor. Smad then associates with Smad4 and is translocated to the nucleus, where it activates target genes. Smad2 and Smad5 have been shown to participate in defining the left-right axis: Binding by activin or TGFβ activates Smad2, whereas Smad5 is activated by BMP ligand binding. Mutations in the Smad2 locus result in embryonic lethality before gastrulation (82, 83). However, analysis of double heterozygotes at the Nodal and Smad2 loci (82), and of post-gastrulation Smad2 mutants that have been rescued by tetraploid embryo complementation, revealed laterality defects (84). Some of the Nodal/Smad2 transheterozygotes had situs anomalies, the most common being transposition of the great arteries, often in association with right pulmonary isomerisms. Smad2 mutants rescued by tetraploid complementation often exhibited delayed or absent embryonic turning, and cardiac looping was leftward or ambiguous (84). These findings suggest that SMAD2 is the component of the TGFβ signaling pathway that relays the “leftness” information provided by an extracellular signal, most probably Nodal, to the nucleus. Smad5 null mice die between 9.5 and 11.5 days of embryogenesis (85, 86). Smad5 /− /− mice are unable to either initiate or complete turning. Defects in heart looping were also observed in these embryos. Lefty-1 expression was usually absent, whereas that of lefty-2, Nodal, and Pitx2 was bilateral (87).

Three transcription factors have been described that are expressed asymmetrically in the lateral plate mesoderm in response to the signaling components of the cascade: the zinc finger protein Snail-Related (SnR), and the homeodomain factors Pitx2 and Nkx3.2. In the chick, SnR is expressed briefly in the primitive streak and subsequently bilaterally in the presumptive cardiac mesoderm with much greater amounts being detected on the right side of the embryo. Expression of cSnR is repressed by SHH and induced by FGF8 (88, 89). Treatment of embryos with antisense SnR oligonucleotides altered the direction of heart looping, with higher concentrations of antisense SnR oligonucleotides causing bilateral Pitx2 expression and disruption of the more posterior morphology (88, 89). In Drosophila, the SnR counterpart, snail, has been proposed to function as a repressor (90), and the observed induction of ectopic Pitx2 expression in the right lateral plate mesoderm after treatment with antisense cSnR oligonucleotides suggests that molecule may have a similar effect on transcription in the chick. However, the inability of ectopic cSnR on the right side to inhibit Pitx2 activation suggests that repression of Pitx2 may be specific to the right side, and that cSnR and Pitx2 have independent effects on heart morphogenesis.
In humans, Pitx2 was initially identified as the homeodomain protein mutated in individuals with Rieger syndrome (97), an autosomal dominant disorder in which affected individuals have defects in ocular, craniofacial, and umbilical development. Pitx2 is expressed in the first branchial arch and its derivatives, the eye, brain, pituitary, heart, somites, limbs, and bone marrow. In addition, Pitx2 is expressed in the left lateral plate mesoderm and asymmetrically during the ontogenesis of several internal organs (29–33, 70). Recently, the Pitx2a isoform has been shown to be asymmetrically expressed in the developing heart field (92), whereas Pitx2c is the isoform expressed asymmetrically in the gut field (92–94). The temporal and spatial expression pattern of Pitx2 suggests that it is a downstream target of the Nodal and Lefty signaling pathways in the lateral plate mesoderm. Indeed, misexpression of Shh, Nodal, or Lefty on the right side of the embryo results in the ectopic expression of Pitx2 in the right lateral plate mesoderm (29–31, 33, 70). In addition, bilateral expression of Pitx2 is capable of altering the normal looping of the cardiac tube as demonstrated by ectopic expression of Pitx2. Finally, the observation that its expression is altered in mouse strains with randomization of organ situs indicates that Pitx2 has a pivotal role in left–right patterning.

Deletion of the Pitx2 locus results in early embryonic lethality associated with several morphological alterations indicative of disruptions in the left-right patterning cascade (94–97). Laterality defects include delayed and incomplete embryonic turning, right pulmonary isomerisms, and a dramatic defect in the ventral body wall closure. Uneven proliferation on the left versus right sides of the embryonic body wall is required for correct axial rotation (98). In Pitx2 mutants, an increase in the number of mesodermal cells in the left lateral body wall at the distal end of the embryo, accompanied by a thickening in the mesodermal component of the amnion in this region, has been observed (94). Although the heart tube appears to loop in the correct direction, subsequent cardiac morphogenesis is abnormal: The right ventricle is hypoplastic, the left atrium is enlarged, and septal defects are present. The apparent malpositioning of the heart may be secondary to the right pulmonary isomerism and incomplete internalization of the visceral organs. In addition, Rieger syndrome–related anomalies are observed in a small percentage of the heterozygotes (95). These findings suggest that Pitx2 regulates the expression of genes required for the organs to complete their morphogenesis and to achieve their correct position relative to the midline.

The homeodomain protein Nkx3.2 is asymmetrically expressed in the anterior left lateral plate mesoderm in the chick embryo beginning at stage 10 (62, 99). By stage 13–14, Nkx3.2 transcripts can also be detected in the right lateral plate mesoderm but in much lower quantities. Lefty-1, Lefty-2, Shh, Caronte, Nodal, and RA are all capable of inducing Nkx3.2 when misexpressed on the right side of the chick embryo (62, 99), whereas ectopic activin or FGF-8 on the left-side of the embryo inhibits Nkx3.2 expression. In addition, overexpression of a truncated form of the activin receptor or placement of beads soaked in a specific FGFR-1 inhibitor on the right side of the embryo has induced Nkx3.2 expression (62, 99). The response of the Nkx3.2 gene to various signaling components in left–right patterning is very similar to what has been observed in the Pitx2 locus. However, Pitx2 and Nkx3.2 expression appear to be activated independently because Pitx2 expression extends more posteriorly, is broader in the dorsoventral axis, and is not observed in the right lateral plate mesoderm. In contrast to the chick, Nkx3.2 in the mouse is predominantly expressed in the right lateral plate mesoderm (99). Homozygous inv embryos, which exhibit a completely penetrant situs inversus phenotype, express high amounts of Nkx3.2/BapX1 in the left lateral plate mesoderm.

Accurate patterning of the left–right axis is required to ensure that the thoracic and visceral organs are correctly positioned relative to the midline. This evolutionarily conserved pattern of organ situs ensures efficient packing into the body cavity and is important for function. During organogenesis, each individual organ must incorporate the information provided by the global left–right patterning cascade into its own morphogenesis program. The discordance of organ situs in the many examples of heterotaxy that have been reported is evidence that each organ interprets and responds to the left–right asymmetry information independently. Several factors have been described that are asymmetrically expressed in one or more of the organs or their primordia and are likely to be involved in specific aspects of development that result in their individual internal asymmetries—and ultimately, in their correct positioning relative to the midline of the embryo. For instance, the extracellular matrix molecule flectin (100) and the basic helix-loophelix (bHLH) transcription factors are asymmetrically expressed in the heart during its development (101). In inv mice, which exhibit complete situs inversus, the expression of all three of these molecules is a mirror image of their patterns in wild-type embryos. Thus, although their expression is regulated in response to left–right signals along the anterior–posterior axis, the integrity of their expression is maintained within the cardiac primordia, such that flectin expression remains associated with the direction of cardiac looping (102), and dHAND and eHAND are expressed in the pulmonary and systemic ventricles, respectively (103).

THE LEFT–RIGHT SIGNALING CASCADE: AN EVOLUTIONARILY CONSERVED MECHANISM?

Substantial progress has been made during the past decade in identifying the molecules responsible for patterning the left–right axis of the embryo and defining their position within a signaling hierarchy. Although the cast of characters responsible for patterning the left and right axes of the embryo is conserved between species, the roles of the individual molecules clearly may differ. The identity of the molecule(s) that becomes asymmetrically distributed in the mouse by way of nodal flow to initiate left–right patterning remains unknown. It will be interesting to discover whether it is related in any way to the factor that initiates asymmetric gene expression in the chick node. Presumably at least one of the functions of this molecule is to regulate the activity or expression of SHH and FGF8, which appear to have
critical early effects in patterning the left-right axis. The conflict in the specific "left" versus "right" patterning activity of FGF8 in mouse versus chick, respectively, will undoubtedly be resolved as our understanding of the intricacies of this patterning cascade improves. Interestingly, this apparent switch in the specific role of Fgf8 does not result in a global inversion of the left and right aspects of the patterning cascade, and a large portion of the remainder of the pathway appears to be evolutionarily conserved. The expression and left–right patterning function of the TGFβ-signaling molecules Nodal and Lefty-2, and at least one of their downstream targets, the transcription factor Pitx2, are similar in all vertebrate model systems. Loss of Nodal, lefty-2, or Pitx2 expression in the left lateral plate mesoderm results in right pulmonary isomerisms, cardiac abnormalities, and inappropriate organ situs, whereas bilateral expression of these genes in the lateral plate mesoderm appears to correlate with left pulmonary isomerisms, cardiac anomalies, and randomized situs. Left- and right-sided aspects of the signaling cascade appear to be essential for maintaining the signal that directs morphological asymmetry. Interfering with earlier events in this developmental pathway appears either to randomize globally or completely reverse organ situs, whereas disrupting later aspects of the cascade may alter the position of only some organs. Understanding the evolutionary diversity of the left–right signaling cascade, and identifying the molecules responsible for the precise morphological movements to generate correct organ situs in response to these signals, will undoubtedly be the focus of this field as we enter the new millennium.

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REFERENCES


