Left-Right Asymmetry and Cardiac Looping: Implications for Cardiac Development and Congenital Heart Disease

IRFAN S. KATHIRIYA AND DEEPAK SRIVASTAVA*

Proper morphogenesis and positioning of internal organs requires delivery and interpretation of precise signals along the anterior-posterior, dorsal-ventral, and left-right axes. An elegant signaling cascade determines left-versus right-sided identity in visceral organs in a concordant fashion, resulting in a predictable left-right (LR) organ asymmetry in all vertebrates. The complex morphogenesis of the heart and its connections to the vasculature are particularly dependent upon coordinated LR signaling pathways. Disorganization of LR signals can result in myriad congenital heart defects that are a consequence of abnormal looping and remodeling of the primitive heart tube into a multi-chambered organ. A framework for understanding how LR asymmetric signals contribute to normal organogenesis has emerged and begins to explain the basis of many human diseases of LR asymmetry. Here we review the impact of LR signaling pathways on cardiac development and congenital heart disease. Am. J. Med. Genet. (Semin. Med. Genet.) 97:271–279, 2000.

KEY WORDS: left-right asymmetry; cardiac looping; cardiac development; congenital heart disease; heterotaxy

INTRODUCTION

The appearance of a bilateral exterior in vertebrates, including humans, disguises dramatic asymmetries of the interior body plan. The heart and vasculature, along with other organs, such as the lungs, stomach, intestines, and brain, show characteristic left-right (LR) asymmetry. Remarkably, the positioning of organs in the normal body plan (situs solitus) is conserved in all vertebrate species studied to date [Fujinaga, 1997]. Although the embryonic body plan is initially symmetric, the first anatomic indication of a more global establishment of LR asymmetry occurs with the rightward looping of the midline heart tube at human embryonic day 23 (mouse embryonic day 8.0–8.5). As the embryo twists in a counterclockwise direction along the rostrocaudal axis, LR asymmetry of other organ systems and the whole body emerges.

Such an undeviating body plan suggests that highly conserved genetic pathways may control determination of the LR axis. In recent years, tremendous progress has been achieved in identifying many of the genes responsible for specifying LR patterning [reviewed in Burdine and Schier, 2000; Capdevila et al., 2000]. Not surprisingly, perturbations in this pathway result in defects of laterality, known as heterotaxy. Translated from its Greek origins to mean “other arrangement,” heterotaxy may occur in inherited or sporadic human diseases of LR abnormalities and manifests as aberrant visceral organ position and/or asymmetry [reviewed in Kosaki and Casey, 1998]. Morphogenesis of the heart appears especially sensitive to aberrations in LR positional information [reviewed in Harvey, 1998; Mercola, 1999]. Alterations in establishment of the LR axis are associated with an assortment of cardiac alignment defects, implying that pathways regulating LR asymmetry affect cardiac development (Table I). In this review, we attempt to integrate important findings from the LR field with clinical observations of aberrant cardiac looping, in an effort to understand better the molecular mechanisms involved in human cardiac laterality defects.

LEFT-RIGHT EMBRYONIC AXIS AFFECTS DIRECTIONALITY OF CARDIAC LOOPING

Elucidation of components in the molecular pathways that establish LR asymmetry has been derived from studies in mouse, chick, frog, and zebrafish (Fig. 1). Many critical steps involve serine/threonine kinase receptors that bind to secreted proteins of the transforming growth factor (TGF)β family [reviewed in Massagué et al., 2000]. From its midline position, Hensen’s node appears to control the establishment of asymmetric LR gene expression throughout the lateral plate mesoderm (LPM) [Levin et al., 1995], which later contributes to most of the visceral organs.

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At Hensen’s node, several genes are expressed asymmetrically (Fig. 1A). On the left in the chick, expression of the morphogen Sonic hedgehog (Shh) becomes restricted to the nodal region and induces perinodal expression of Nodal [Levin et al., 1995; Pagán-Westphal and Tabin, 1998], a member of the TGF-β signaling family. Transfer of this “leftness” positional information from the node to the periphery in the LPM is mediated by a Cerberus/Dan-related secreted protein, Caronte (Car) [Rodriguez-Esteban et al., 1999; Yokouchi et al., 1999; Zhu et al., 1999]. As Car expression extends to the left LPM, Car directly antagonizes a subclass of TGF-β family members known as bone morphogenetic proteins (BMPs), relieving BMP-mediated suppression of Nodal and possibly lefty-2 in the LPM (Fig. 1B). Similar to other Cerberus family members, Car also can inhibit Nodal by direct binding, providing a negative

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### TABLE I. Mutations Affecting Laterality and Cardiac Looping

<table>
<thead>
<tr>
<th>Gene</th>
<th>Organism</th>
<th>Laterality and cardiac phenotype</th>
<th>Reference</th>
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<tr>
<td>Node component</td>
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<tr>
<td>iv/Lrd</td>
<td>Mouse</td>
<td>Heterotaxy, randomized heart looping</td>
<td>Supp et al. [1997, 1999]</td>
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<td>KIF3A</td>
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<td>Heterotaxy, randomized heart looping</td>
<td>Marszalek et al. [1999]; Takeda et al. [1999]</td>
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<td>KIF3B</td>
<td>Mouse</td>
<td>Heterotaxy, randomized heart looping</td>
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<td>Signaling molecule</td>
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<tr>
<td>Shh</td>
<td>Mouse</td>
<td>Randomized heart looping and abnormal heart position</td>
<td>Meyers and Martin [1999]</td>
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<td>FGF-8</td>
<td>Mouse</td>
<td>Right pulmonary isomerism, randomized heart looping</td>
<td>Meyers and Martin [1999]</td>
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<tr>
<td>Activin receptor llb</td>
<td>Mouse</td>
<td>“Asplenia syndrome,” randomization of heart position, right atrial isomerism, TGA, VSD, ASD</td>
<td>Oh and Li [1997]</td>
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<tr>
<td>ACVR2B</td>
<td>Human</td>
<td>Various situs abnormalities</td>
<td>Kosaki et al. [1999b]</td>
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<tr>
<td>Cryptic</td>
<td>Mouse</td>
<td>Right pulmonary isomerism, randomized cardiac looping, TGA, AVS defects</td>
<td>Gao et al. [1999]; Yan et al. [1999]</td>
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<td>CRYPTIC</td>
<td>Human</td>
<td>Various situs abnormalities</td>
<td>Bamford et al. [2000]</td>
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<td>Lefty-1 (LEFTYB)</td>
<td>Mouse</td>
<td>“Polysplenia syndrome,” left thoracic isomerism, left atrial isomerism</td>
<td>Meno et al. [1998]</td>
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<td>LEFTYA (lefty-2)</td>
<td>Human</td>
<td>Various situs abnormalities</td>
<td>Kosaki et al. [1999a]</td>
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<td>Nodal+/− Smad2+/−</td>
<td>Mouse</td>
<td>Right pulmonary isomerism, TGA</td>
<td>Nomura and Li [1998]</td>
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<tr>
<td>Cyclops (Nodal)</td>
<td>Zebrafish</td>
<td>Midline defects, including cyclopia, randomized cardiac looping</td>
<td>Chen et al. [1997]; Schilling et al. [1999]; Bisgrove et al. [2000]</td>
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<td>Transcription factor</td>
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<td>Ptx2</td>
<td>Mouse</td>
<td>Right pulmonary isomerism, common AVC</td>
<td>Gage et al. [1999]; Kitamura et al. [1999]; Lin et al. [1999]</td>
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<td>ZIC3</td>
<td>Human</td>
<td>X-linked situs abnormalities</td>
<td>Gebbia et al. [1997]</td>
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<td>No tail</td>
<td>Zebrafish</td>
<td>Midline defects, randomized heart looping</td>
<td>Danos and Yost [1996]; Chen et al. [1997]; Schilling et al. [1999]; Bisgrove et al. [2000]</td>
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<td>Floating head</td>
<td>Zebrafish</td>
<td>Midline defects, randomized heart looping</td>
<td>Danos and Yost [1996]; Chen et al. [1997]; Schilling et al. [1999]; Bisgrove et al. [2000]</td>
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<td>Smad5</td>
<td>Mouse</td>
<td>Randomized heart looping, embryos fail to turn</td>
<td>Chang et al. [1999, 2000]</td>
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<td>Unknown function</td>
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<td>Momo</td>
<td>Zebrafish</td>
<td>Midline defects, randomized heart looping</td>
<td>Odenthal et al. [1996]; Bisgrove et al. [2000]</td>
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<td>Inv/inversin</td>
<td>Mouse</td>
<td>Situs inversus (mirror-image reversal)</td>
<td>Lowe et al. [1996]; Mochizuki et al. [1998]; Morgan et al. [1998]</td>
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FGF, fibroblast growth factor; TGA, transposition of the great arteries; VSD, ventricular septal defect; ASD, atrial septal defect; AVS, atrioventricular septum; AVC, atrioventricular canal; Shh, Sonic hedgehog.
feedback loop to maintain precisely the levels of Nodal activity. Subsequent left-sided expression of Nodal induces expression of a bicoid-type homeodomain protein, Pitx2, in the LPM. Expression of Pitx2 continues during asymmetric organ development along the left side of the heart tube, gut, and lungs [Logan et al., 1998; Piedra et al., 1998; Ryan et al., 1998; Yoshioka et al., 1998; Campione et al., 1999; Essner et al., 2000; Schweickert et al., 2000]. In this fashion, LR information is transmitted from the node to the visceral organs.

On the right side, an inferred activin-like activity inhibits Shh expression [Levin et al., 1995], while fibroblast growth factor (FGF)-8 is up-regulated near the node [Boettger et al., 1999], where it inhibits Car expression [Rodriguez-Esteban et al., 1999; Yokouchi et al., 1999]. Consequently, unrestrained BMP-mediated pathways repress right-sided Nodal expression and up-regulate the snail-related zinc finger transcription factor (SnR) [Isaac et al., 1997] in the right LPM. Together, these signals establish the LR axis and convey the positional information necessary for body situs and asymmetric organogenesis.

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Studies in the mouse have shown that ciliary projections on the node beat in a vortical movement to create a “nodal flow” that may displace molecules functioning as left determinants to the left of the node [Nonaka et al., 1998; Okada et al., 1999; Takeda et al., 1999]. It is believed that determination of the LR axis in mice is initiated by ciliary beating that establishes asymmetric gene expression near the node, while a midline barrier, possibly lefty-1, maintains the accumulation of morphogens on the left side (Fig. 1A). The inversus viscerum (iv/iv) mouse strain displays situs solitus (normal position), situs inversus (mirror-image reversal), or situs ambiguous, a condition in which the arrangement of LR organization is uncoupled in visceral organs, similar to heterotaxy syndrome. Randomization of heart, lung, and gut asymmetry reflects a lack of coordination by the LR signaling pathways and often is associated with abnormal organogenesis. Expression of molecular markers for the LR pathway, such as Nodal [Supp et al., 1997] and Pitx2 [Piedra et al., 1998; Ryan et al., 1998; Campione et al., 1999] in the iv/iv mouse is normal, reversed, bilateral, or absent, suggesting a malfunction in the biasing mechanism that distinguishes left from right.

The iv gene product encodes for left-right dynein (Lrd), which is an axonal-type dynein that acts as a force-generating component in cilia that is expressed at many sites, including nodal cells [Supp et al., 1997, 1999]. Mice lacking Lrd or genes that encode other components of the ciliary motor, such as KIF3A or KIF3B, have immotile nodal cilia and thus fail to generate initial LR asymmetry around the node [Nonaka et al., 1998; Marszalek et al., 1999; Okada et al., 1999; Supp et al., 1999; Takeda et al., 1999]. With such evidence, a molecular model can begin to explain the clinical finding that situs abnormalities correlate with ciliary dysfunction in immotile cilia syndrome, also known as Kartagener syndrome [Afzelius, 1976].

Intriguing evolutionary questions have been raised by differences in chick and mouse with regard to the establishment of LR asymmetry. It is not yet known whether there are motile cilia in the chick node. However, the initial nodal asymmetry of FGF-8 and Shh is reversed in mice compared with chick, as are some downstream events, such as asymmetric expression of Nkx3.2 [Meyers and Martin, 1999; Rodriguez-Esteban et al., 1999; Schneider et al., 1999]. Despite the disparity in early events, LR asymmetry of downstream events involving Nodal and Pitx2 appear to be conserved in all species studied to date [Yost, 1999]. The discordance of early LR asymmetry among species has yet to be explained, but it may reflect the presence of undiscovered pathways regulating transmission of LR information from the node to the LPM.

In the murine model of situs inversus (inv), nearly all of the inv mutants show complete reversal of the LR axis as it relates to organ asymmetry and position. Molecular analyses show Nodal and Pitx2 expression along the right rather than the left LPM, suggesting an inversion in LR signaling [Lowe et al., 1996; Yoshioka et al., 1998; Campione et al., 1999]. In the clinical setting, patients that have situs inversus have a well-coordinated reversal of LR visceral asymmetry and thus have a low incidence of defects in organogenesis, which is similar to the findings in the inv mouse model (Fig. 2D). Although the inv gene product, inversin, is known, its function remains a mystery [Mochizuki et al., 1998; Morgan et al., 1998].

Like the phenotype of the inv/inv mouse, the phenotype of the majority of patients with LR defects consists of situs ambiguous (visceral heterotaxy) and possible defects in almost all aspects of cardiogenesis. Often, one side predominates; patients show signs of either bilateral right-sidedness, a condition known as “asplenia syndrome,” or bilateral left-sidedness, a condition known as “polysplenia syndrome.” Commonly, one side of a visceral organ is duplicated, and this is referred to as an isomerism. Several examples exist in which components of the LR signaling cascade are disrupted in mice, resulting in heterotaxy.

Targeted disruption of Pitx2 in the mouse results in right pulmonary iso-
Cascades regulating left-right (LR) asymmetry. Early asymmetric gene expression around the node (A) results in activation or repression of Sonic hedgehog (Shh)—or fibroblast growth factor (FGF)-8—dependent pathways on the right or left (ventral view). Early roles of Shh and FGF8 are reversed in mouse and chick, as indicated in color-coded fashion. Leftward flow of morphogens (green dots) by nodal cilia establishes the asymmetric gradient around the node in mice. Expression of Lefty-1 near the midline may serve as a barrier to maintain left-sided asymmetry of morphogens. At later stages of organogenesis, LR asymmetric information at the node is transferred to the lateral plate mesoderm (LPM) by Caronte. Caronte relieves bone morphogenic protein (BMP) inhibition on the left, initiating a cascade of events that culminates in expression of Pitx2 in the left LPM and on the left side of the heart tube (B). Consequently, a “leftness” (green) signal appears to be propagated actively to overcome a default “rightness” (yellow) program [adapted from Capdevila et al., 2000].
merism [Gage et al., 1999; Kitamura et al., 1999; Lin et al., 1999]. Although the hearts of most Pitx2 mutant embryos loop in the correct direction, the morphologic features of the heart appear abnormal, perhaps as a result of an anomalous process of looping. It remains possible that a low percentage of Pitx2 mutants may display reversal of cardiac looping. Mice mutant for activin receptor IIb have a predominance of right-sidedness, similar to asplenia syndrome, and heart defects, which include randomization of position, transposition of the great arteries (TGA), ventricular septal defect, and atrial septal defect [Oh and Li, 1997]. Similarly, mice deficient for cryptic, the extracellular cofactor of Nodal, show signs of right pulmonary isomerism, randomization of cardiac looping, TGA, and atrioventricular septal defects [Gaio et al., 1999; Yan et al., 1999]. Finally, targeted disruption of lefty-1 in mice leads to left rather than right thoracic isomerism, as in polysplenia syndrome [Meno et al., 1998]. This evidence suggests that in the absence of coordinated LR decisions, malalignments of ventriculo-arterial or atrioventricular connections may occur.

Studies in zebrafish and Xenopus have augmented our understanding of the common themes involved in vertebrate LR asymmetry. For example, in Xenopus, as early as the one-cell stage

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**Figure 2.** Normal and abnormal cardiac morphogenesis associated with left-right signaling. A: As the linear heart tube loops rightward with inner curvature (ic) remodeling and outer curvature (oc) proliferation, the endocardial cushions (dark blue) of the inflow (green) and outflow (light blue) tracts become adjacent to each other. Subsequently, the atrioventricular septum shifts to the right, while the aortopulmonary trunk shifts to the left. ta, truncus arteriosus; at, atrium; rv, right ventricle; lv, left ventricle. B: The inflow tract is divided into the right (ravc) and left (lavc) atrioventricular canal by the atrioventricular septum (asterisk). The outflow tract, known as the truncus arteriosus, becomes the aortopulmonary trunk (apt) upon septation. ra, right atrium; la, left atrium. C: Ultimately, the left atrium and right atrium are aligned with the left ventricle and right ventricle, respectively. The left ventricle and right ventricle become aligned with the aorta (ao) and the pulmonary artery (pa), respectively, after 180° rotation of the great vessels. mv, mitral valve; pv, pulmonary valve; tv, tricuspid valve. D: If the determinants of the left-right axis are reversed coordinatedly, then a condition known as situs inversus results. E: If the aortopulmonary trunk fails to shift to the left, a condition known as double-outlet right ventricle (DORV) results, in which the right ventricle is aligned with both the aorta and the pulmonary artery. vsd, ventricular septal defect. F: Likewise, if the AVS fails to shift to the right, both atria communicate with the left ventricle in a condition known as double-inlet left ventricle (DILV). G: Transposition of the great arteries (TGA) results if the aortopulmonary trunk fails to twist, resulting in communication of the right ventricle with the aorta and the left ventricle with the pulmonary artery.
human orthologs of ZIC3, a zinc finger transcription factor, is a causative factor in LR asymmetry that include abnormal directionality of heart looping. In contrast, the right atrium appears to develop from right-sided atrial progenitors, while the left atrium develops from those on the left [reviewed in Srivastava and Olson, 2000]. Mouse models mutant for activin receptor IIb [Oh and Li, 1997] or lefty-1 [Meno et al., 1998] show right or left atrial isomerism, respectively, suggesting responsiveness by the left and right atria to LR signals.

The process of cardiac looping intricately establishes the relative positions of cardiac chambers and their vascular connections (Fig. 2A–C). The inflow and outflow tract cushions, swellings of extracellular matrix that remodel into valve tissue, become positioned adjacent to one another by folding of the heart tube (Fig. 2A). Subsequently, the left and right atria must align with the appropriate ventricular chambers, and each ventricle must connect with the aorta or pulmonary artery. This process is mediated by morphogenesis of the atrioventricular septum (AVS), which divides the inflow tract, known as the common atrioventricular canal (AVC), into a right and left AVC (Fig. 2B). As the AVS shifts to the right to lie above the ventricular septum, the AVCs follow and consequently become aligned over their respective ventricles. In conjunction, the common outflow tract, known as the truncus arteriosus, septates to become the aortopulmonary trunk. As it shifts to the left to situate itself over the AVS, the vascular trunk twists 180° to position the aorta properly over the left ventricle and the pulmonary artery correctly over the right ventricle (Fig. 2C). In this manner, the cardiovascular system is converted from a circuit in series to a parallel circulation, in preparation for terrestrial life away from the womb.

Molecular modifications in cellular proliferation, transformation, migration, and death are thought to be involved in the process of looping, but the relative contributions of these cellular mechanisms remain unknown. The inner curvature of the looping heart tube appears to be remodeled, while the outer curvature actively proliferates. A phenomenon known as myocardialization may explain why trabeculations in the ventricles are found on the outer curvature while the inner curvature remains smooth [reviewed in Mjaatvedt et al., 1999]. Along the inner curvature, cardiomyocytes evacuate and migrate to the cushions, where they invade and muscularize without proliferating, resulting in relocation of myocardium from the inner curvature to the cushions. Mice with trisomy 16 (syntenic to parts of human chromosome 21 and 22) have defects in myocardialization, such that the myocardium of the inner curvature cannot be removed or remodeled in the absence of cushion development, resulting in defects in the process of looping [Webb et al., 1996].

Genes differentially expressed along the outer or inner curvatures of the looping heart tube have been discovered and may provide a molecular basis for their morphologic differences. Genes encoding atrial natriuretic factor (ANF) and SERCA2a, the sarcoplasmic reticulum calcium pump necessary for cardiac excitation and contraction, are expressed in the outer curvature of the ventricles.
and atria but are absent in the inner curvature [Christoffels et al., 2000]. Additionally, two members of the basic helix-loop-helix family of transcription factors, dHAND and eHAND, are expressed in a complementary and ventricle-specific fashion, predominantly along the outer curvature of the heart [Biben and Harvey, 1997; Srivastava et al., 1997; Thomas et al., 1998]. This evidence suggests that intrinsically properties of the two surfaces may be central to cardiac looping.

Aberrations in the process of cardiac looping may result in many congenital heart diseases involving alignment defects. After the heart tube has initiated cardiac looping, any arrest or delay in the positioning of the AVS or the conotruncal region may result in malalignment of the inflow and outflow tracts with the ventricles (Fig. 2D–G). For example, if the conotruncus is unable to migrate to the left, the right ventricle communicates with the aorta and pulmonary artery, resulting in a condition known as double-inlet left ventricle (DILV) (Fig. 2E). Likewise, the AVS may fail to move to the right, resulting in a condition known as double-outlet right ventricle (DORV) (Fig. 2E). Likewise, the AVS may fail to move to the right, resulting in a condition known as double-inlet left ventricle (DILV), in which the left ventricle communicates with both the left and right AVC (Fig. 2F). Genetic evidence for this has been discovered from embryos containing a targeted disruption for nodal/TGF-β pathway [reviewed by Schier and Shen, 2000], which display laterality defects and an outflow alignment defect that is limited to TGA [Nomura and Li, 1998].

Together, these clinical observations and molecular evidence hint at the interaction of pathways that regulate the process and the directionality of cardiac looping. Indeed, some matrix proteins in the chick, such as flectin [Tsuda et al., 1998], are expressed asymmetrically along the linear heart tube and may play a role in the mechanical forces guiding cardiac looping. The discovery that Pitx2 is expressed asymmetrically in the LPM and on the left side of developing organs provides the first transcriptional inroad into understanding how LR signals might affect organogenesis. In the heart, targets of Pitx2 on the left side of the heart tube may interact with cascades governing cardiac development to promote appropriate direction and remodeling of the looping heart tube.

**FUTURE DIRECTIONS**

We have witnessed a remarkably rapid pace of discovery over the past five years in the field of LR asymmetry. An intricate LR biasing mechanism appears to be initiated early during embryogenesis, well before any evidence of organogenesis. However, many questions remain. Why are the early events of LR asymmetry less conserved across species than later events? What intermediate steps remain undiscovered between early node signals and subsequent LPM gene expression? What are the mechanisms through which LR asymmetric gene expression exerts its influence on individual organs? Ultimately, how does disruption of LR signals result in defects of organogenesis? Undoubtedly, these and other similar questions will be explored in the coming years and may provide a deeper understanding of human diseases associated with anomalies of laterality.

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