Diversity and convergence in the mechanisms establishing L/R asymmetry in metazoa

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Abstract

Differentiating left and right hand sides during embryogenesis represents a major event in body patterning. Left–Right (L/R) asymmetry in bilateria is essential for handed positioning, morphogenesis and ultimately the function of organs (including the brain), with defective L/R asymmetry leading to severe pathologies in human. How and when symmetry is initially broken during embryogenesis remains debated and is a major focus in the field. Work done over the past 20 years, in both vertebrate and invertebrate models, has revealed a number of distinct pathways and mechanisms important for establishing L/R asymmetry and for spreading it to tissues and organs. In this review, we summarize our current knowledge and discuss the diversity of L/R patterning from cells to organs during evolution.

Introduction

The first mutation affecting the whole body plan was isolated a century ago and was shown to invert shell coiling in a small aquatic snail (*Lymnaea peregra*) [1,2]. Despite this early finding and important work describing genetic and cellular aspects of L/R asymmetry [3–11], the first molecular study of L/R asymmetry was described only recently, showing for the first time asymmetric expression of the *nodal* gene in vertebrates [12]. A possible reason for this lag is the fact that in contrast to A/P and D/V asymmetries, laterality is not obvious at first sight, when looking at the external body shape, with snail shell coiling being an exception. Indeed, despite looking mostly bilaterally symmetrical, metazoa also differentiate along the "invisible" L/R axis, leading to asymmetric positioning of unique organs, such as the heart, liver and stomach, and asymmetrical morphogenesis of bilateral ones, as for example the lung and brain.

In addition, L/R asymmetry controls the looping of tubular organs (heart tube, gut, and other ducts) toward one direction. Laterality is thus essential for the correct arrangement of visceral organs in the abdomen and thorax, but is also essential for the asymmetric morphogenesis, hence the function, of the heart and brain, for example. Clinical studies led to an estimation of 1/5,000–1/10,000 humans suffering from L/R defects (*situs inversus*, heterotaxia, and isomerism), being responsible for a number of complex congenital heart defects, misrotation of the intestine, and spontaneous miscarriage. Furthermore, L/R asymmetry defects, which often originate from ciliopathies, are associated with polycystic renal disease, Kartagener and Ivemark syndromes, and others.

L/R asymmetry is therefore essential, and outstanding questions remain to be addressed to understand how body shape and function are established during evolution. What is, or what are, the origin(s) of L/R asymmetry? Where and when does it take place in the embryo? Are there any conserved features among metazoa and how did L/R asymmetry establishment evolve in metazoa (Sidebar A)?

A specificity of L/R asymmetry is the fact that it has to be coordinated with the other two—A/P and D/V—body axes and thus is established relative to and after them as a "secondary" axis. This important notion was summarized by Brown and Wolpert in their elegant F-molecule model [13]. The incremental/two-step establishment of body patterning is particularly interesting, as it implies that L/R asymmetry establishment depends on mechanisms that integrate existing 2D positional information. Over the last few years, several studies using different model organisms helped to identify unique mechanisms at play during the establishment of L/R asymmetry. Although a variety of mechanisms have been discovered, fascinating similarities between quite distant phyla are emerging. On the following pages, we will discuss the various mechanisms and synthesize common principles of L/R asymmetry establishment in vertebrates and invertebrates.

Vertebrate embryonic node and Nodal flow in L/R patterning

A well-established model for the determination of the body situs in several vertebrate species is that of the Nodal flow occurring at the

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A/P	anterior/posterior
Abd-B	abdominal-B
ASEL, ASER	left–right asymmetric bilateral sensory neurons in *C. elegans*
D/V	dorsal/ventral
dvl	*dishevelled-like*
FGF	fibroblast growth factor
GSK3	glycogen synthase kinase 3
Heterotaxia	also *situs ambiguus*, uncoordinated placing of the internal organs
Isomerism	situation in which both sides of the body adopt the same fate
iv	*inversus viscerum*
L/R	left–right
LPM	lateral plate mesoderm
myoID	*myosin ID*
PCP	planar cell polarity
PH	Pleckstrin Homology
Pitx2	*paired-like homeodomain transcription factor 2*
Pkd1l1	*polycystic kidney disease-like 1*
Pkd2	*polycystic kidney disease 2*
Shh	sonic hedgehog
situs inversus	inverted placing of the internal organs
situs solitus	normal placing of the internal organs
TGF-β	transforming growth factor beta
vangl	*Van Gogh-like*

late-gastrulation-neurulation stage in the mouse node and node-like structures of other animals (Posterior Notochordal Plate in rabbit, Kupffer's Vesicle in zebrafish, Gastrocoel Roof Plate in Xenopus) [14–16].

The Nodal flow model is best described in mouse, which serves as the model paradigm; hence, we focus in the following on the description of the data obtained in mouse. The node is a transitory structure located on the ventral side of the embryo at the end of the developing notochord (Fig 1A). The node is a cavity covered by a monociliated epithelium-like monolayer of cells, which appears decisive for proper lateralization [17]. Indeed, when the node cilia are missing, mice show abnormal L/R patterning with random lateralization, that is, both the normal *situs solitus* and the inverted *situs inversus* are observed. This is for instance the case in mice mutant for the Kif3A or Kif3B subunits of the kinesin-II complex, a microtubule motor essential for proper ciliogenesis and maintenance of the cilium. In these mutants, cilia fail to assemble [18,19].

However, it is not merely the presence of these cilia that is important, but rather their motility. Indeed, *inversus viscerum* (*iv*) mutant mice, in which the cilia are present but immotile, show similar randomized lateralization phenotypes [20,21]. *iv* encodes the L/R dynein, another microtubule motor essential for node cilia motility [20]. Node cilia rotate clockwise, thereby producing a leftward flow of extra-embryonic fluid, which appears to determine the directionality of embryo lateralization [18,19,22,23]. Cilia have been known for some time to be important for lateralization [24], but their role in the production of the Nodal flow was only recently described [18] (Fig 1A). Impairing the flow genetically (with mutant mice) or experimentally (by increasing the viscosity of the medium) leads to L/R patterning defects [25]. When the node cilia are missing or immotile, the Nodal flow is abolished
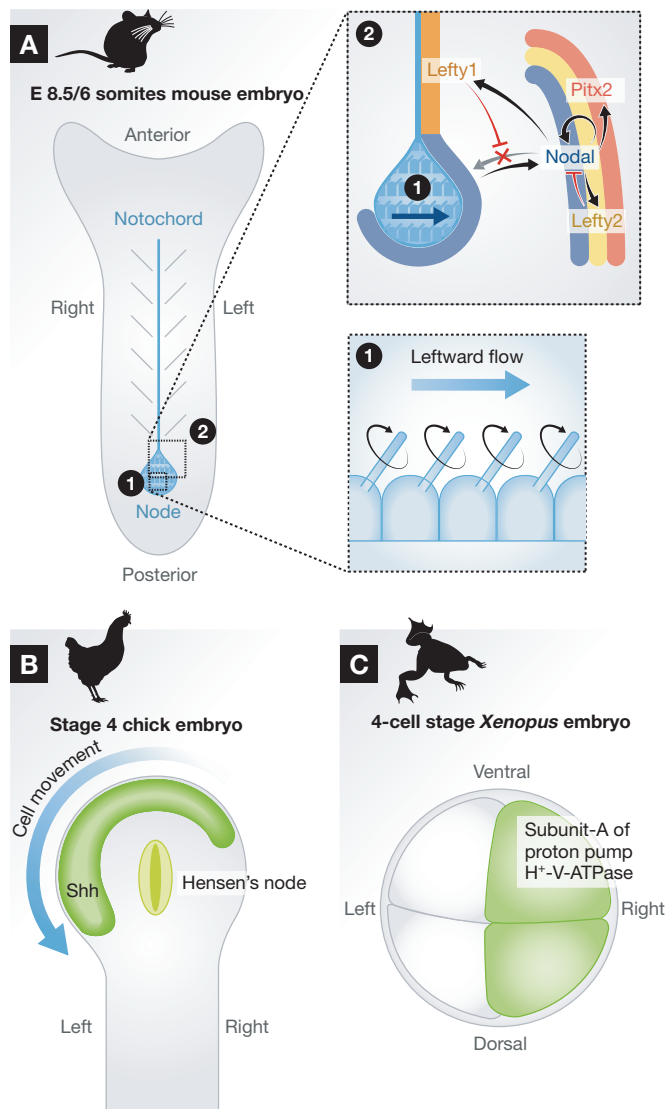

Figure 1. Left/Right determination in vertebrates.
(A) Schematic depiction of a E8.5 mouse embryo. Nodal is expressed around the node. Nodal flow (i) leads to stronger expression of Nodal on the left side (ii) and in the Lateral Plate Mesoderm (LPM) where it positively regulates its own expression by a positive feedback loop. Nodal also activates expression of the homeobox transcription factor Pitx2 and of the TGF-β homologues Lefty2 and Lefty1 in the LPM near the notochord. Lefty1/2 antagonize Nodal diffusion to the right side of the embryo and ultimately shut down Nodal signaling. Pitx2 expression is self-maintained and induces left-sided morphogenesis of the LPM. (B) Schematic depiction of a stage 4 chick embryo's primitive streak and Hensen's node. The leftward movement of cells from the right of Hensen's node induces the asymmetric remodffieling of the node's morphology as well as asymmetric gene expression patterns (e.g. Shh, green) due to the intermingling of cells with different genetic programs [57,58]. (C) *Xenopus* embryo at the 4-cell stage shows right-sided enrichment in subunit-A of the proton pump H⁺-V-ATPase, whose activity is necessary for proper lateralization of the animal. Interestingly, this early L/R asymmetric localization appears to be sensitive to actin but not microtubule depolymerization [60].

and the L/R *situs* is consequently randomized. Interestingly, the restoration of an artificially generated leftward Nodal flow is sufficient to reinstate normal L/R patterning of mutant mice [25]. Conversely, in wild-type mice, superimposition of an artificial

rightward Nodal flow is able to override normal patterning and leads to inversion of the axis, demonstrating the importance of the flow in this process [25].

The normal mouse node is thought to comprise between 200 and 300 motile cilia, nevertheless only a couple of them seem to be required for normal lateralization [26]. This precision was achieved through thorough analysis of the phenotype of mutant mice, in which ciliogenesis was strongly impaired but that nevertheless retain some cilia at the node. This is for instance the case in mice mutant for the Rfx3 transcription factor necessary for ciliogenesis. The discovery that only two motile cilia—but not one—wherever their position in the node, were sufficient to trigger normal L/R patterning questions the sensitivity of the Nodal flow signal or the existence of a on/off effect of the flow [26]. Remarkably, the generation of a small difference or initial bias between the left and right sides by the Nodal flow appears to be sufficient to be turned into robust asymmetry [27]. Similar analyses of flow dynamics in various genetic conditions showed that in zebrafish, the flow generated by thirty motile cilia or more reliably predicts the future laterality of the animal [28]. Interestingly, the authors revealed distinct sensitivities of different organs to the flow. These observations could account for heterotaxia in conditions in which the flow is compromised but not abolished.

How is the information provided by the Nodal flow implemented for asymmetric morphogenesis, and how does the Nodal-signaling cascade initiate left-sided morphogenesis? Originally detected on both sides of the node, Nodal expression is reinforced on the left side by the Nodal flow. Nodal, a TGF-β family member, diffuses to the LPM surrounding the node where it activates a positive feedback loop inducing its own expression, as well as those of Lefty2 and Pitx2 in the LPM and that of Lefty1 around the midline [29] (Fig 1A). Lefty1 and Lefty2 molecules are monomeric TGF-β family members that compete with Nodal signaling in the extracellular medium. The expression of Lefty1 at the midline antagonizes the Nodal produced on the left side of the embryo LPM, thus preventing the diffusion of Nodal activity to the right side and subsequent ectopic left-sided development [30,31]. Consistently, *nodal* mutants display right-sided characteristics on both sides (right isomerism), whereas both sides of *Lefty1* mutants show left-sided characteristics [27,29,30]. Downstream of Nodal signaling is the homeodomain-bearing transcription factor Pitx2. *Pitx2* expression once activated by Nodal remains expressed in the LPM. Its expression dictates left-sided morphogenesis of the asymmetric organs, thus presaging the development of morphological asymmetries of the body [32–35].

These data show the importance of the flow generated by the node cilia in locking the directionality of the L/R axis. However, cilia rotating around their axis (from their base to their tip) should produce a vortex without any clear directionality and not the laminar flow that is observed experimentally. How can the clockwise rotation of the cilia produce a leftward flow? The answer is twofold. First, the apical surface of the node cells forming the embryonic cavity appears to be convex, and second, their basal body (that anchors the cilium in the cell) is asymmetrically positioned. In the node epithelium, the cilia basal bodies are not positioned in the middle of the apical side but at the posterior end [36,37]. These two factors lead to a posterior tilt of the cilia, which in turn leads to an effective stroke toward the left side and an ineffective recovery stroke toward the right side, thereby creating the observed leftward flow [36–38].

How is this coordinated localization of the node cell basal bodies from their initial central apical location to the posterior attained across the node epithelium? A well-known example of the uniform orientation of all cells in the plane of an epithelium is that of PCP. PCP was first described in *Drosophila* ommatidia and wing bristles, whose coordinated orientation was shown to genetically depend on so-called PCP genes [39]. Proper L/R axis establishment is also impaired in mice mutant for the PCP genes *dvl* and *vangl,* due to the randomization of the cilia position at the surface of the node pit cells. Thanks to PCP signaling, all node cells have their cilium basal body located similarly at the posterior end of their apical domain and can thus participate in the generation of the coordinated Nodal flow [40–43]. Interestingly, the positioning of the cilia basal bodies also depends on actin cytoskeleton remodeling, as the cooperation of the PCP core protein Vangl2 and the actin-severing protein Cofilin1 appears to be important in this process [44]. In *vangl2;cofilin1* double mutant mice, the basal body fails to migrate posteriorly and remains centrally located leading to L/R patterning randomization [44]. Taken together, these data link the generation of the extra-embryonic Nodal flow to the intracellular cell cytoskeleton organization and A/P axis.

Several questions remain, as for example, how does the Nodal flow induce organism lateralization and subsequent asymmetric morphogenesis? How is the Nodal flow sensed? It is now clear that in addition to the node pit cell cilia, a second population of cilia located on the crown cells around the node is crucial for sensing the flow. To date, two not mutually exclusive hypotheses are debated, the first chemical and the other mechanical (for review see [36,45]). The former asserts that a morphogen gradient is established by the Nodal flow and sensed by the perinodal crown cells. Nodal Vesicular Parcels are membrane-sheathed vesicles originating from the node cell that are released in an FGF-dependent fashion [46]. These Nodal Vesicular Parcels are suggested to be transported by the Nodal flow and to produce a putative gradient of molecules, such as Shh and retinoic acid [18]. This hypothesis needs to gather firmer experimental confirmation in order to be corroborated. The latter hypothesis, the mechanical one, claims that the signal carried by the Nodal flow is actually the pressure that is sensed by the sensory cilia of the perinodal crown cells [21].

Whichever the mechanism, it has been shown that the perception of the Nodal flow requires the Ca^{2+} channel encoded by the *Pkd2* and *Pkd1l1* genes [47,48]. Interestingly, this complex appears to be required solely in the perinodal crown cells for proper L/R establishment. In *Pkd2* null-mutant mice, Pkd2 expression was reintroduced by transgenesis in the perinodal crown cells but not in the node pit cells. This localized expression was sufficient to restore normal L/R patterning [49]. Consistently, mice with normal *Pkd2* expression, in which cilia are absent from node pit cells and only present in the perinodal crown cells, are able to respond to an artificial flow and trigger proper left-sided morphogenesis [19]. This suggests that the Pkd2 and Pkd1l1 complex could be responsible for the detection of the Nodal flow and possibly for the resultant Ca^{2+} signal observed on the left side of the node [47,48,50]. However, how this Ca^{2+} signal impacts on Nodal expression and the subsequent signaling cascade remains to be resolved.

The Nodal flow model is very popular as it provides a comfortable mental frame to link cell polarity to structural chirality and

ultimately to organism lateralization, but additional mechanisms could be at play during vertebrate L/R axis establishment. Although no early L/R asymmetry has yet been described in mouse, one study found that blastomere repositioning at the 4- and 8-cell stages affects the stereotypical embryonic axial rotation occurring days later [51]. Furthermore, the left–right dynein encoded by the *iv* locus and known for its role in L/R asymmetry (as mentioned above) has recently been implicated in the process of chromatid segregation [52], thus opening the way for a "chromatid segregation" model hypothesizing a L/R asymmetric imprinting of the chromatin from the zygote first cell division on [53]. In addition, recent investigations suggest that a Nodal-independent mechanism, relying on actin polymerization and myosin II activity, could control heart-looping lateralization in zebrafish [54]. Other Nodal flow independent mechanisms of L/R patterning in vertebrates and invertebrates are discussed in more detail below.

Ion flux and left–right determination in vertebrates

Several vertebrate species with a node-like structure do not seem to rely on the Nodal flow for their L/R axis determination. In chick for instance, the homologous structure, the Hensen's node, differs from the mouse node. The mouse or rabbit node is formed of mesodermal pit cells whose motile cilia produce a flow [36,55]. In the chick, on the other hand, Hensen's node cells are endodermal cells with shorter and immotile cilia [56]. Interestingly, the chick node itself becomes morphologically asymmetric and adopts a leftward tilt due to cellular rearrangements, cell migration, and interactions with the surrounding tissues (Fig 1B) [57,58]. This observation does not seem to be a peculiarity of the chick, or of non-mammalian vertebrates, as it was also reported in the pig embryo [58]. Remarkably, these cell migration properties, which precede asymmetric Nodal expression by several hours, directly depend on the L/R program and are downstream of the earlier H^+/K^+ ATPase activity [58].

A whole body of work has shown the involvement of ion pumps of various kinds in L/R patterning at the earliest stages of development. Initially identified through pharmacological screening for the effect of drugs on lateralization, ion pumps and ion channels such as H^+/K^+-ATPase, H^+-V-ATPase, or Na^+/K^+-ATPase, were found to possess asymmetric localizations and activities at developmental stages prior to the "Node" and as early as the first cleavages in several vertebrate species (Fig 1C) [59–61]. The asymmetric expression of these pumps and channels on one side of the embryo is thought to generate a localized ion flow creating steady differences in pH and transmembrane voltage between left and right sides of the embryo. These pH or electrical gradients are thought to orient lateralization or to mediate the local concentration of small signaling molecules (for review see [14,16]). Indeed, when the ion pump or channel activity is missing, the resultant phenotype is often heterotaxia, that is, an uncoordinated L/R axis [59–61]. Interestingly, some data indicate that the initial asymmetry of these ion pumps during early development depends on the correct organization of the cell cytoskeleton [60]. To our knowledge, no data on whether ion pumps, channels or other mechanisms preceding the Nodal flow stage could be at play in mouse L/R asymmetry establishment are yet available. Taken together, it appears that in several vertebrate species, L/R asymmetry is established at different times of development and via different mechanisms.

Left–right asymmetry determination in non-vertebrate deuterostomes

Several of the actors and mechanisms found in vertebrate L/R determination appear to be conserved in non-vertebrate deuterostomes without Node-like structures, such as the ascidian *Ciona intestinalis* and *Halocynthia roretzi* or the echinodermata sea urchin. The *C. intestinalis* larva possesses two asymmetrically located sensory pigment spots near the brain as well as an asymmetric gut [62]. Similarly to the aforementioned vertebrates, Nodal signaling is detected on the left side of *C. intestinalis* and leads to the expression of the Pitx2 homologue, which in turn directs left-sided morphogenesis [62]. Interestingly, H^+/K^+ ATPase activity also appears to act shortly before Nodal expression in *C. intestinalis* and its perturbation affects the left-sided expression of the *Pitx2* homologue, indicating the requirement for the ion channel in *C. intestinalis* L/R patterning as well [62]. In *H. roretzi*, another ascidian, Nodal signaling is also detected on the left side of the embryo for L/R morphogenesis. However, in *H. roretzi*, Nodal expression depends on embryo-wide movements that bring the embryo epidermis and the vitelline membrane in contact. Indeed, a recent study shows that Nodal expression originates from this contact [63]. Interestingly, the contact zone is consistently fixed through a cilium-driven stereotypical rotation of the neurula-stage embryo, called the "neurula rotation" [63]. These data, once more linking Nodal and ciliary function, suggest that cilia could act in more than one way for L/R determination. Finally, in the sea urchin pluteus larva, the adult rudiment (the progenitor tissue for the future sea urchin) forms on one side of the mesodermal tissues [64,65]. Here, Nodal and H^+/K^+ ATPase activities are also involved in L/R patterning [65,66]. But there is a twist to it, as in sea urchin, Nodal is not a left side marker or inducer but is instead found to be expressed on the larval right side, where it prevents left-sided development of the adult rudiment [65,66].

Left–right asymmetry determination in invertebrates

Although they do not all possess asymmetrically positioned organs, most bilaterian animals show some kind of internal L/R asymmetry. Bilateria is a big clade containing the Deuterostomes and Protostomes phyla. All the aforementioned species belong to the Deuterostomes, yet the Protostomes (usually referred to as "invertebrates") are key to understand the basis of L/R patterning both at the morphological and at the functional level [14,67]. Among those, studying three different genera, snails of the *Lymnaea* genus, the *Caenorhabditis elegans* nematode, and the *Drosophila melanogaster* fruit fly, led to some major advances in our understanding of L/R asymmetry, which are discussed below.

Lymnaea *snails*

In snails, L/R asymmetry can be seen in the asymmetric positioning of organs such as the gonad or renal organ but is most evident in the coiling of their shell, whose direction is firmly controlled. There

are snail species with dextral coiling, others with sinistral coiling of their shell. Yet, snails with inverted shell coiling can naturally occur within a strain and prove invaluable to the study of L/R axis determination and patterning. In snails, both *nodal* and *Pitx* homologues are asymmetrically expressed during embryogenesis. Their expression is localized to the right side of dextral snails and to the left in sinistral snails and is important for the normal asymmetric production of the shell. Indeed, treatment with a general chemical inhibitor of the TGF-β superfamily (to which Nodal belongs) led to some individuals with non-coiled shells, which could suggest a loss of asymmetry [68]. A possible downstream effector of *Nodal/Pitx* signaling guiding the asymmetric growth of the shell could be the morphogen Dpp, another TGF-β family member. Indeed, *Dpp* expression appears to predict shell coiling in several species [69].

What controls the asymmetric *Nodal/Pitx* expression in snails? The exact symmetry-breaking event is unknown, but it appears to happen at the earliest stages of embryo development. At the 8-cell stage, the blastomere arrangement appears chiral. The four micromeres on top have their "axis" slightly shifted to one side compared to the bottom macromeres (Fig 2A). This "spiral" positioning of the blastomeres occurs at the third cleavage and predicts the coiling direction of the shell. It is thus found to the left in sinistral species and to the right in dextral ones [68,70,71]. Yet, the situation is strikingly different between variants of a given species, at least for the first stages. Until the 8-cell stage, the *situs inversus* embryos have all their blastomeres aligned, thus lacking the top micromere tilt of the *situs solitus* embryos of the same species [70,71]. But from the 8-cell stage onwards, an inversed tilt happens and the *situs solitus* and *situs inversus* individuals appear to be mirror images. These observations raised the possibility that two distinct mechanisms could be at play to control the dextral and sinistral fates [70]. Furthermore, micromanipulations of the blastomere arrangement during the third cleavage (leading to the 8-cell stage) can impose lateralization on the embryos (Fig 2A). Indeed, inversing the normal tilt of the blastomeres in *situs solitus* embryos or restoring a spiral blastomeric arrangement in *situs inversus* ones triggers the coiling of the shell of the resulting adults in the direction imposed by the manipulation, as well as *Nodal* and *Pitx* asymmetric expression during development [70]. These results indicate the crucial importance of the early asymmetric mechanisms at play at the third cleavage stage for L/R axis establishment. Interestingly, treatment of 4-cell stage embryos with the microtubule depolymerizing agent nocodazole does not affect proper L/R development, whereas treatments with actin depolymerizing agents such as latrunculin A or B at the same four-cell stage do impair snail lateralization, indicating the importance of the actin cytoskeleton in this process [71].

In spite of these indications, the molecular mechanisms regulating snail chirality remain unknown. Genetic experiments have shown that shell chirality depends on a single gene [72,73]. Taking advantage of the naturally occurring sinistral individuals of *Lymnaea peregra*, geneticists performed crossing experiments and found that shell directionality depends on a single locus of the maternal genome [73]. Furthermore, injection of dextral egg cytoplasm into sinistral eggs was sufficient to induce normal dextral development, whereas the injection of sinistral egg cytoplasm into dextral eggs had no effect, indicating that the dextral allele is dominant over the sinistral one [73]. Interestingly, phylogeny modeling has shown that determination of shell coiling by a single gene is

evolutionary conserved [74] and that it could reflect an adaptive prey/predator response to snake asymmetric mandibles [75]. However, the exact gene that controls dextral coiling has not yet been identified, despite several attempts [72]. And thus, the nature of this maternally inherited and dominant dextral cytoplasmic factor, which is present in the egg and likely acts on the actin cytoskeleton during the first developmental cleavages, remains unknown.

Caenorhabditis elegans

Caenorhabditis elegans is a popular model system, for which the stereotypical developmental fate of each of the one thousand or so cells has been precisely mapped. *Caenorhabditis elegans* possesses many LR asymmetric features as well as asymmetrically positioned organs, such as the gonad, spermatheca, or vulva (for review on L/R patterning in *C. elegans* see [76,77]). Although the exact symmetry-breaking event during *C. elegans* development is unknown, the genetic regulation controlling asymmetric morphogenesis has been carefully dissected.

The dextral positioning of blastomeres occurring at the 4- to 6-cell stage transition is the first apparent sign of L/R asymmetry. This process has been heavily used to study early L/R patterning [76,78,79]. During the transition from the 4- to 6-cell stage, the anterior and posterior dorsal blastomeres slightly turn to the right, thus orientating the mitotic spindle rightward (Fig 2B). Upon cytokinesis, this asymmetric division leads to the rightward daughter cells to be positioned posteriorly relative to the leftward ones, the whole embryo thus adopting a dextral orientation (Fig 2B). The bias in the direction of the mitotic spindle appears to originate from the earliest stage of embryonic development. The one-cell embryo stereotypically rotates by 120° always in the same direction prior to the first mitosis. This process relies on the organization of the actin cytoskeleton, as depletion of the WAVE-Arp2/3 complex or of the CYK-1 Formin homologue impairs embryo rotation and *C. elegans* laterality, thus revealing the existence of an actin-based intrinsic chirality [80]. This initial chirality seems to be transmitted to the astral microtubules of the spindle, through the cortical G-alpha protein encoded by the *gpa-16* gene. Loss of *gpa-16* G-alpha protein activity leads to random lateralization of the 6-cell stage blastomere [81]. Consistently, disruption of the spindle orientation process similarly results in the randomization of 6-cell blastomere positioning [81,82]. These data suggest that these mechanisms are used to orient the mitotic spindle in order to fix consistent L/R development. Among these mechanisms, the non-canonical Wnt signaling pathway has been suggested to act on the cytoskeleton and thereby control blastomere spindle orientation [83,84]. From stage 12 onwards, a series of Notch inductions controls L/R patterning [85]. Indeed, after the asymmetric blastomere division at the 6-cell stage, a first Notch induction instructs asymmetric L/R patterning [80]. Thus, the original L/R asymmetries in spindle orientation are at the basis of later L/R patterning in worms [80,86].

Finally, the *C. elegans* brain shows two kinds of neuronal L/R asymmetries. First is the stochastic expression of GFP in a reporter line in a set of two neurons that are thus termed "On/Off" [87,88]. Through calcium signals between these two neurons, only one of the pair expresses the odorant receptor gene *str-2* [88]. This process rather corresponds to anti-symmetry than to proper stereotyped L/R
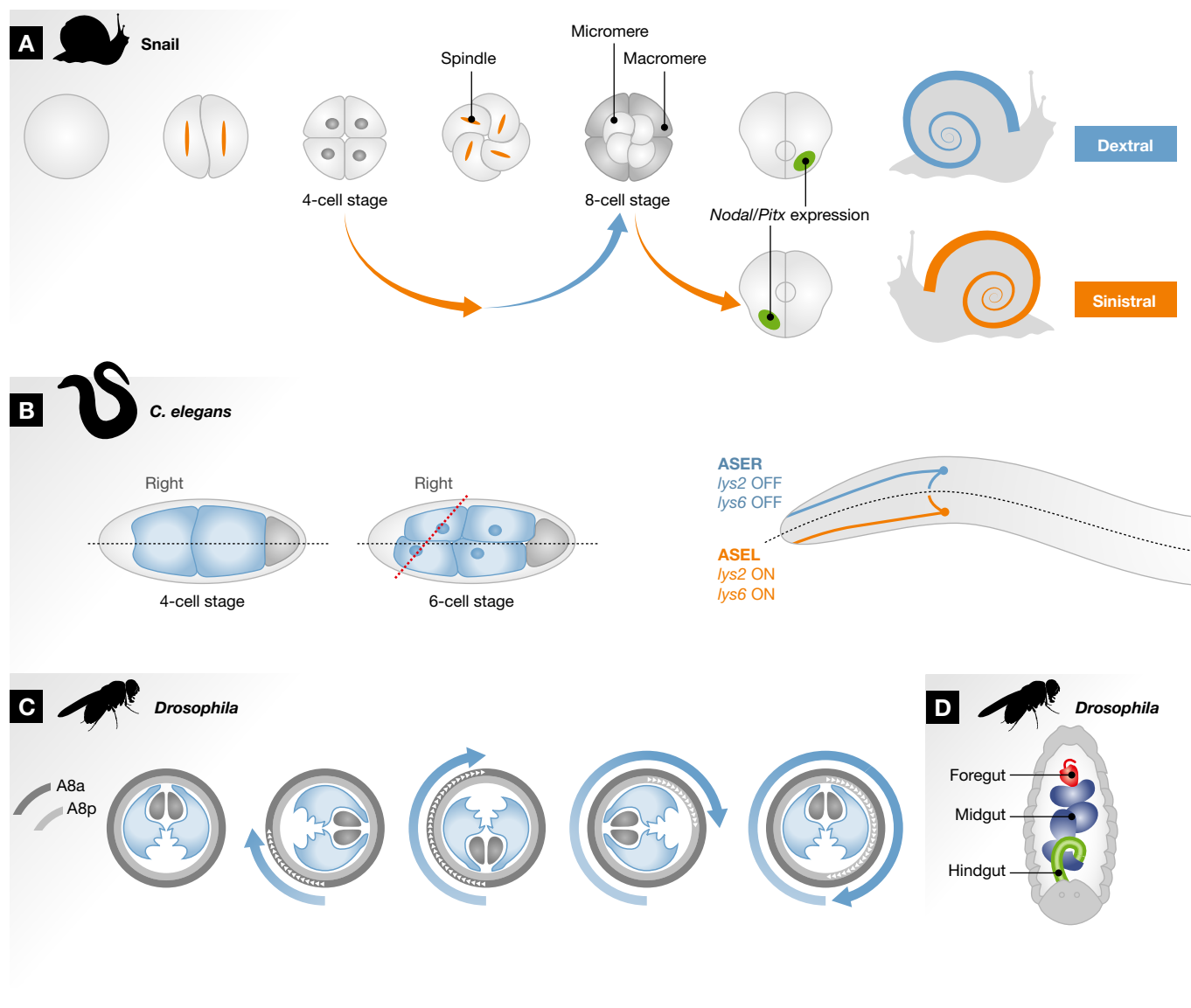
Figure 2.   Left/right determination in Protostomes.
(A) In snails, L/R asymmetry is manifested in the coiling of the shell. The direction of this coiling depends on the orientation of the first two cell cleavages. The asymmetric spatial arrangement of the blastomeres leads to the spiral orientation of the spindles. Whereas forced inversion at the 2- to 4-cell stage causes only a temporal L/R perturbation, mended at the 4-cell stage, forced inversion at the 4- to 8-cell stage results in a permanent inversion of the L/R axis highlighted by asymmetric Nodal and Pitx expression (green spot). (B) The first clear asymmetric marker in *Caenorhabditis elegans* is the dextral placement of blastomeres during the 4–6 cell stage transition. The anterior cell and the posterior cell slightly spin so that the mitotic spindle orients rightward, with the result that the midline reorients slightly dextrally. This early asymmetry is propagated later on; one example is the appearance of the functionally asymmetric ASEL/ASER neurons, controlled by the specific expression of *lys2* and *lys6* genes. (C) Terminalia looping in *Drosophila* depends on the rotations of two independent rings, the A8a and the A8p, each contributing 180° (white arrowheads on A8a and A8p) to the 360° rotation (blue arrowheads). Although they are in close proximity, the direction of rotation of each of these rings, dextral or sinistral, is independent of each other and only depends on the presence and absence of the dextral determinant MyoID. (D) The gut of the *Drosophila* embryo is divided in three parts, foregut (red), midgut (blue), and hindgut (green), each displaying a complex L/R asymmetry pattern.

asymmetry. Second is the stereotyped L/R asymmetry of the neuron pair ASEL/ASER (Fig 2B). Although the ASEL/ASER fate also depends on the 6-cell stage blastomere asymmetry, their future differentiation is determined at the 24-cell stage through two rounds of Notch inductions that leave a L/R mark on the postmitotic neurons [89,90]. Recent work identified the nature of the L/R marks and found that a miRNA, encoded by the *lsy-6* locus, induced chromatin de-compaction in the neuron committed to the ASEL fate [91,92].

Drosophila melanogaster

In all the model systems reviewed so far, the animal L/R axis appears to be established sequentially from an initial symmetry-breaking event, yet in *Drosophila* the various L/R organs seem to be able to individually lateralize owing to the existence of L/R organizing centers [93,94 and González-Morales N *et al*, in preparation]. Furthermore, it is a striking feature of *Drosophila* that a reset of the lateralization can occur at metamorphosis (for review on L/R patterning in *Drosophila* see [95]). In *Drosophila*, most L/R research

has been performed on the lateralization of two organs at two different times of development: first, the dextral looping of the embryonic hindgut during embryogenesis, and second, the dextral 360° rotation of the male terminalia and the associated coiling of the spermiduct during metamorphosis (Fig 2C and D, [95,96]). The dextral orientation of these organs, as well as that of the other *Drosophila* L/R asymmetric organs, depends on the activity of a single gene: *myosin ID* (*myoID*). When *myoID* activity is missing, *Drosophila* L/R asymmetry is inverted, thus revealing the activity of an underlying sinistral pathway [94,97]. Interestingly, in some of these organs, L/R organizers could be identified in which MyoID activity was exclusively required for normal dextral development of the organ [94 and González-Morales N *et al*, in preparation]. Using temporally and spatially controlled genetic tools, it was shown that L/R establishment of the embryonic hindgut and terminalia is independent and happens at two distinct developmental times [94,97,98].

Further thorough analysis of *myoID* expression yielded unanticipated results. Indeed, in the L/R organizer controlling terminalia rotation, MyoID is expressed in two distinct cell rows [94]. Interestingly, these two MyoID expression domains each correspond to the two independent rings contributing to the 360° terminalia rotation. Selective depletion of *myoID* activity in one, the other, or both domains shows that each cell ring contributes 180° to the rotation and that they behave as two genetically independent mini-L/R organizers. Consequently, when *myoID* activity is present, the ring rotates dextrally by 180° and by 180° sinistrally when *myoID* activity is missing. These data open startling evolutionary perspectives which could explain the observed diversity in terminalia rotation in diptera, through the appearance and later duplication of a 180° L/R unit [99].

Recently, the Hox transcription factor Abd-B was identified as the upstream regulator of L/R determination in *Drosophila* (Fig 3A). *Abd-B* and other Hox genes are key to establish A/P identity [100], nevertheless this new function in L/R patterning appears to be separate. Upon depletion of *Abd-B* activity in the embryonic hindgut or the male terminalia L/R organizer, loss of *myoID* expression is observed [93]. Nevertheless, unlike *myoID* loss of function, *Abd-B* depletion does not result in an inverted asymmetric development of the L/R axis but in the loss of asymmetry leading to a symmetric development of the organs [93]. Remarkably, restoring MyoID expression is sufficient to rescue this phenotype indicating that Abd-B controls the expression of the symmetry-breaking factor, the dextral determinant MyoID. Furthermore, *Abd-B* depletion in a *myoID* null, and so sinistral, background similarly yields flies developing symmetrically, showing that a genuine sinistral pathway, also under the control of Abd-B, exists (Fig 3A) [93]. These data suggest that factors involved in L/R axis establishment might be able to "read" the A/P axis.

Molecularly, the dextral determinant MyoID is a type I unconventional myosin, a one-headed, monomeric actin-based motor, that is very well conserved in evolution [94,97,101]. Type I myosins comprise three domains: an N-terminal single-headed motor domain coupled to a C-terminal tail via an alpha-helical neck [102,103]. The motor domain binds actin and hydrolyses ATP. The neck contains a number of IQ domains and binds light chains acting as a lever-arm, thus transmitting the conformational changes that occur in the motor domain after ATP hydrolysis [104,105]. Finally, the tail
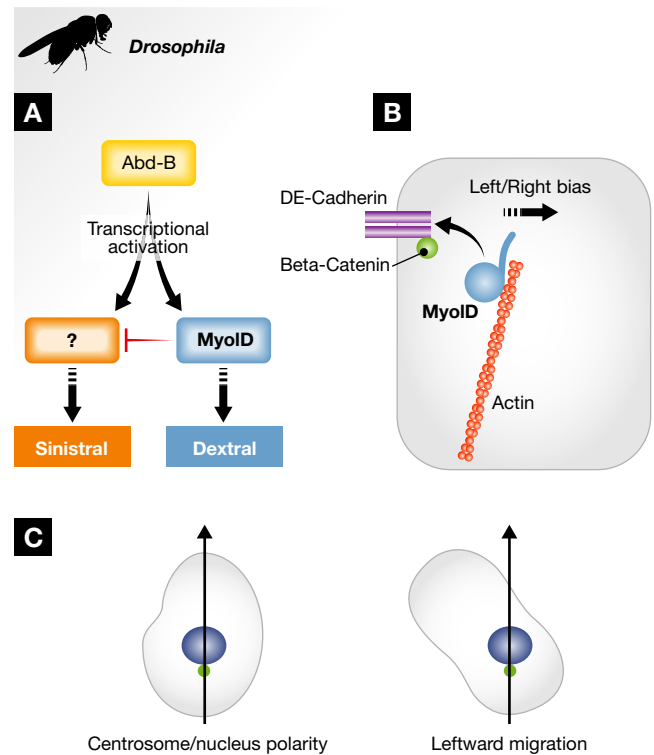

Figure 3. Genetic and cellular determination of *Drosophila* L/R asymmetry.

Schematic depiction of genetic and cellular aspects of *Drosophila* lateralization. (A) The wild-type, or "dextral", orientation depends on the activity of MyoID (Blue). Dextral determination is dominant over sinistral determination (Red), which only becomes apparent in *myoID* null flies. Interestingly, Abd-B (Yellow) controls the expression and/or activity of the two opposite pathways. In *Abd-B* depleted flies, the L/R organs develop symmetrically [93]. To date, the putative sinistral counterpart to MyoID is still unknown. (B) In the cells of the L/R organizer, MyoID (blue) binds to cortical actin (red) and needs to associate with the adherens junction components E-cadherin (yellow) and β-catenin (green) at the apical membrane for proper L/R determination [94,109]. (C) Several lines of cultured vertebrate cells orient themselves according to their nucleus–centrosome axis (arrow) and are thus able to migrate in a L/R asymmetric manner.

domain is thought to interact with cargos and binds membrane phospholipids through its Pleckstrin Homology domain, a positively charged lipid-binding region [106,107].

How does MyoID act during L/R determination? Interestingly, MyoID activity appears to be required only for a short time to induce a dextral bias [94]. To date, the exact mechanism of MyoID action remains unknown, but the actin-binding head domain appears to be central for L/R patterning [98]. Additionally, in the cells of the organizer, MyoID requires the adherens junction components β-catenin and E-cadherin as well as a properly organized cortical actin cytoskeleton (Fig 3B) to induce dextral L/R development [94,97,98,108,109]. In the epithelium of the embryonic hindgut, MyoID has been shown to cell-autonomously bias cell chirality and induce membrane bending [108]. Interestingly, computer simulations showed that mild membrane bending in each cell is sufficient to induce a complete dextral loop of the hindgut [108]. MyoID-dependent membrane bending appears to be mediated by E-cadherin, as membranes in *E-cadherin* null mutants do not

bend [108]. Taken together, these data suggest that L/R morphogenesis could originate from asymmetric membrane tension generated by a MyoID/E-cadherin complex. Interestingly, unlike in the absence of E-cadherin or β-catenin when no consistent orientation is seen, in the absence of MyoID cell membranes of the hindgut still bend, but this time in the opposite direction [108,109]. These observations suggest that the sinistral factor(s), whose activity is only apparent in the absence of MyoID, is also able to induce an orientated cell membrane bias.

Innate cellular chirality

As mentioned above, asymmetric traits are not specific to multicellular structures but can also appear at the single cell level. Indeed, numerous cell types exhibit chiral structures, orientated movements as well as chiral behaviors [110–113]. These observations argue that intracellular elements might underlie L/R asymmetry determination. This idea, termed the "intracellular model", has been around for some time and proposes that the origin of asymmetry in the body plan relies on intracellular structures and in particular the actin cytoskeleton [16]. Supporting this model is the fact that in cultured migrating cells, a clear 3D cell polarity can be seen. In addition to the first two axes, rear-front and top-bottom, a third one, drawn from the center of the nucleus to that of the centrosome, demonstrates clear cell chirality and corresponds loosely to the direction followed by these cells during their migration [113]. However, when cultured in contact with a repeated pattern, cells consistently migrate with a clear bias toward the left side of this third axis (Fig 3C), strongly suggesting the existence of an intracellular bias present in each individual cell [110,112,113].

The cell chirality depends on the cell cytoskeleton. Disrupting microtubule integrity leads to randomization, revealing the need for an intact microtubule cytoskeleton for this leftward bias [113]. Disruption of the actin cytoskeleton instead leads to the "inversion of the cell L/R axis" that is, a rightward bias in cell migration [110]. Consistently, the expression of constitutively active GSK3 similarly inverts the cell "L/R axis". The cells now polarize to the right of the nucleus-to-centrosome axis. These data suggest that GSK3 could act as a link between the unknown original chiral template and the cytoskeleton sensing the spatial cues and orienting cell polarity [113]. These data, obtained in vertebrate cells, are reminiscent of the link between the actin cytoskeleton and L/R patterning in *Drosophila, C. elegans* or the *Lymnaea* snails, suggesting a conserved mechanism. Furthermore, they also support the existence of a sinistral factor, as cell or organismal orientation can be consistently inverted and not simply randomized. However, a diversity of L/R orientations exists in cultured cells with some cell types having a dextral bias, others a sinistral one and some with no bias at all [111,112]. To conclude, cell culture experiments revealed the crucial role of actin dynamics for internal cell chirality and suggest that both dextral and sinistral L/R patterning might originate from intracellular polarity.

Indeed, several pieces of evidence obtained from studies of type I myosins and actin dynamics support the idea that L/R asymmetries can be created *de novo* from basic cell components [114]. Type I myosins, to which *Drosophila* MyoID belongs, are members of the myosin superfamily of actin-based motors and are found in most eukaryotic cells [115,116]. In vertebrates, eight type I myosins (myosin I a–h) are found, whereas only two members exist in *Drosophila* (myosin ID and IC) [117,118]. Recent work, using *in vitro* binding of murine MyoIc to actin, revealed that
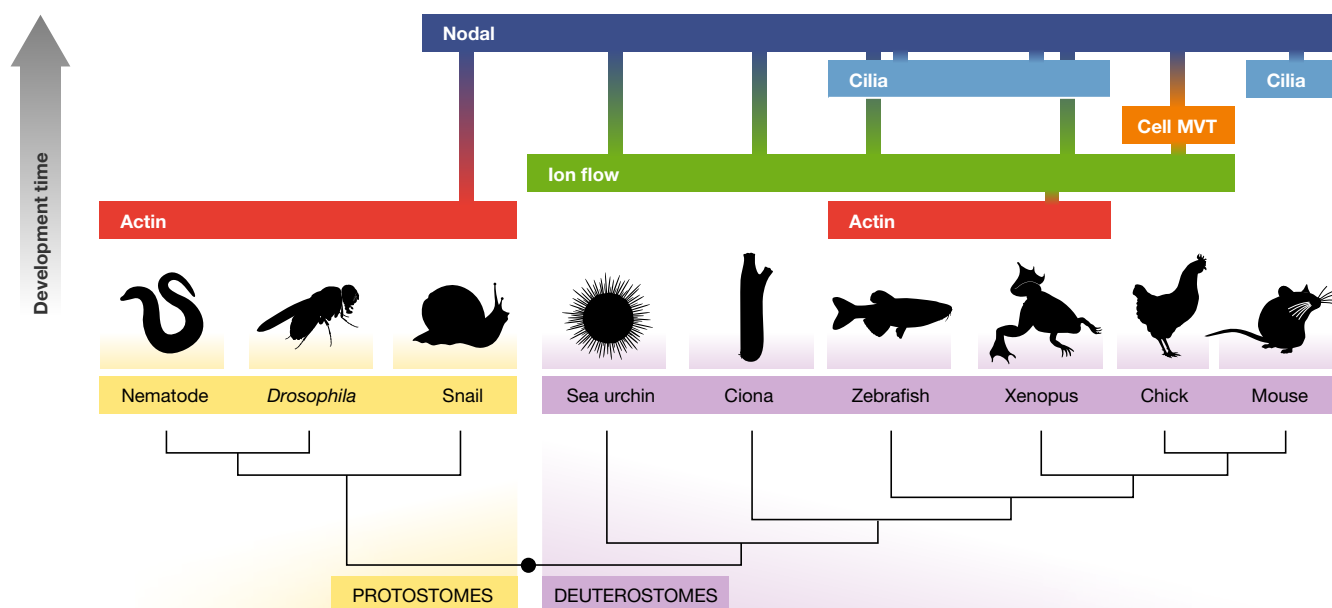

Figure 4. L/R asymmetry in metazoa: diversity and convergence.
Common and divergent principles of L/R asymmetry establishment in the model systems discussed in this review (see text for details). Species are aligned along a phylogenetic tree discerning Protostomes (yellow) and Deuterostomes (purple). The mechanisms breaking symmetry (actin-based: red; ion flow: green; cell movement (Cell MVT): orange; cilia-based Nodal flow (Cilia): light blue) are vertically aligned along the developmental time (DVPT TIME) at which they act (early, down; later, up). The direct link between a mechanism and a subsequent one or ultimately to the *Nodal*-signaling pathway (Nodal, dark blue) is indicated by the color gradient.

MyoIc can asymmetrically guide motility, leading to actin filaments that curl counterclockwise [114]. Importantly, this generation of asymmetric motility appears to be a property of MyoIc and not a universal characteristic of myosin I motors, since neither murine MyoIa nor Ib are able to generate a similar asymmetric actin movement [114]. Although it is not directly stated, the head domain seems to be responsible for this feature, which is consistent with the fact that, *in vivo*, the L/R activity of *Drosophila* MyoID also appears to depend on its head domain [98]. The finding that specific myosins can make actin fibers chiral are the earliest described signs of asymmetry somehow related to L/R patterning.

Taken together, it appears that from all the model systems discussed, Nodal flow is rather an exception in L/R axis establishment (Fig 4). Evolutionarily, it could correspond to a refinement that was added to earlier mechanisms happening at the cellular level. The conserved involvement of fundamental cellular elements such as ion channels or cytoskeletal components may point to common ancestral L/R asymmetry mechanisms. Additionally, they allow for the generation of a theoretical model for how, from core molecules at the cellular level, such as the actin cytoskeleton, L/R patterning may be created in metazoans.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1.   Boycott AE, Diver C (1923) On the inheritance of sinistrality in *Limnaea peregra*. *Proc R Soc Lond B, Contain Papers Biol Charact* 95: 207 – 213

2.   Gurdon JB (2005) Sinistral snails and gentlemen scientists. *Cell* 123: 751 – 753

3.   Danos MC, Yost HJ (1995) Linkage of cardiac left-right asymmetry and dorsal-anterior development in Xenopus. *Development* 121: 1467 – 1474

4.   Fujinaga M, Baden JM (1991) A new method for explanting early postimplantation rat embryos for culture. *Teratology* 43: 95 – 100

5.   Hoyle C, Brown NA, Wolpert L (1992) Development of left/right handedness in the chick heart. *Development* 115: 1071 – 1078

6.   Hummel KP, Chapman DB (1959) Visceral inversion and associated anomalies in the mouse. *J Hered* 50: 9 – 13

7.   Layton WM, Layton MW, Binder M, Kurnit DM, Hanzlik AJ, Van Keuren M, Biddle FG (1993) Expression of the IV (reversed and/or heterotaxic) phenotype in SWV mice. *Teratology* 47: 595 – 602

8.   Schreiner CM, Scott WJ Jr, Supp DM, Potter SS (1993) Correlation of forelimb malformation asymmetries with visceral organ situs in the transgenic mouse insertional mutation, legless. *Dev Biol* 158: 560 – 562

9.   Stalsberg H (1969) The origin of heart asymmetry: right and left contributions to the early chick embryo heart. *Dev Biol* 19: 109 – 127

10. Yokoyama T, Copeland NG, Jenkins NA, Montgomery CA, Elder FF, Overbeek PA (1993) Reversal of left-right asymmetry: a situs inversus mutation. *Science* 260: 679 – 682

11. Yost HJ (1991) Development of the left-right axis in amphibians. *Ciba Found Symp* 162: 165 – 176; discussion 176–181

12. Levin M, Johnson RL, Stern CD, Kuehn M, Tabin C (1995) A molecular pathway determining left-right asymmetry in chick embryogenesis. *Cell* 82: 803 – 814

13. Brown NA, Wolpert L (1990) The development of handedness in left/right asymmetry. *Development* 109: 1 – 9

14. Speder P, Petzoldt A, Suzanne M, Noselli S (2007) Strategies to establish left/right asymmetry in vertebrates and invertebrates. *Curr Opin Genet Dev* 17: 351 – 358

15. Hirokawa N, Tanaka Y, Okada Y, Takeda S (2006) Nodal flow and the generation of left-right asymmetry. *Cell* 125: 33 – 45

16. Vandenberg LN, Levin M (2013) A unified model for left-right asymmetry? Comparison and synthesis of molecular models of embryonic laterality. *Dev Biol* 379: 1 – 15

17. Lee JD, Anderson KV (2008) Morphogenesis of the node and notochord: the cellular basis for the establishment and maintenance of left-right asymmetry in the mouse. *Dev Dyn* 237: 3464 – 3476

18. Nonaka S, Tanaka Y, Okada Y, Takeda S, Harada A, Kanai Y, Kido M, Hirokawa N (1998) Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. *Cell* 95: 829 – 837

19. Takeda S, Yonekawa Y, Tanaka Y, Okada Y, Nonaka S, Hirokawa N (1999) Left-right asymmetry and kinesin superfamily protein KIF3A: new insights in determination of laterality and mesoderm induction by kif3A$^{-/-}$ mice analysis. *J Cell Biol* 145: 825 – 836

20. Supp DM, Witte DP, Potter SS, Brueckner M (1997) Mutation of an axonemal dynein affects left-right asymmetry in inversus viscerum mice. *Nature* 389: 963 – 966

21. McGrath J, Somlo S, Makova S, Tian X, Brueckner M (2003) Two populations of node monocilia initiate left-right asymmetry in the mouse. *Cell* 114: 61 – 73

22. Supp DM, Brueckner M, Kuehn MR, Witte DP, Lowe LA, McGrath J, Corrales J, Potter SS (1999) Targeted deletion of the ATP binding domain of left-right dynein confirms its role in specifying development of left-right asymmetries. *Development* 126: 5495 – 5504

23. Okada Y, Nonaka S, Tanaka Y, Saijoh Y, Hamada H, Hirokawa N (1999) Abnormal nodal flow precedes situs inversus in iv and inv mice. *Mol Cell* 4: 459 – 468

24. Kartagener H (1935) Bronchiektasien bei situs viscerum inversus. *Schweiz Med Wocshenschr* 65: 782 – 784

25. Nonaka S, Shiratori H, Saijoh Y, Hamada H (2002) Determination of left-right patterning of the mouse embryo by artificial nodal flow. *Nature* 418: 96 – 99

26. Shinohara K, Kawasumi A, Takamatsu A, Yoshiba S, Botilde Y, Motoyama N, Reith W, Durand B, Shiratori H, Hamada H (2012) Two rotating

cilia in the node cavity are sufficient to break left-right symmetry in the mouse embryo. *Nat Commun* 3: 622

27. Nakamura T, Mine N, Nakaguchi E, Mochizuki A, Yamamoto M, Yashiro K, Meno C, 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

28. Sampaio P, Ferreira RR, Guerrero A, Pintado P, Tavares B, Amaro J, Smith AA, Montenegro-Johnson T, Smith DJ, Lopes SS (2014) Left-right organizer flow dynamics: how much cilia activity reliably yields laterality? *Dev Cell* 29: 716−728

29. Brennan J, Norris DP, Robertson EJ (2002) Nodal activity in the node governs left-right asymmetry. *Genes Dev* 16: 2339−2344

30. Meno C, Shimono A, Saijoh Y, Yashiro K, Mochida K, Ohishi S, Noji S, Kondoh H, Hamada H (1998) Lefty-1 is required for left-right determination as a regulator of lefty-2 and nodal. *Cell* 94: 287−297

31. Yamamoto M, Mine N, Mochida K, Sakai Y, Saijoh Y, Meno C, Hamada H (2003) Nodal signaling induces the midline barrier by activating nodal expression in the lateral plate. *Development* 130: 1795−1804

32. Logan M, Pagan-Westphal SM, Smith DM, Paganessi L, Tabin CJ (1998) The transcription factor Pitx2 mediates situs-specific morphogenesis in response to left-right asymmetric signals. *Cell* 94: 307−317

33. Piedra ME, Icardo JM, Albajar M, Rodriguez-Rey JC, Ros MA (1998) Pitx2 participates in the late phase of the pathway controlling left-right asymmetry. *Cell* 94: 319−324

34. Ryan AK, Blumberg B, Rodriguez-Esteban C, Yonei-Tamura S, Tamura K, Tsukui T, de la Peña J, Sabbagh W, Greenwald J, Choe S et al (1998) Pitx2 determines left-right asymmetry of internal organs in vertebrates. *Nature* 394: 545−551

35. Yoshioka H, Meno C, Koshiba K, Sugihara M, Itoh H, Ishimaru Y, Inoue T, Ohuchi H, Semina EV, Murray JC et al (1998) Pitx2, a bicoid-type homeobox gene, is involved in a lefty-signaling pathway in determination of left-right asymmetry. *Cell* 94: 299−305

36. Okada Y, Takeda S, Tanaka Y, Izpisua Belmonte JC, Hirokawa N (2005) Mechanism of nodal flow: a conserved symmetry breaking event in left-right axis determination. *Cell* 121: 633−644

37. Nonaka S, Yoshiba S, Watanabe D, Ikeuchi S, Goto T, Marshall WF, Hamada H (2005) *De novo* formation of left-right asymmetry by posterior tilt of nodal cilia. *PLoS Biol* 3: e268

38. Cartwright JH, Piro O, Tuval I (2004) Fluid-dynamical basis of the embryonic development of left-right asymmetry in vertebrates. *Proc Natl Acad Sci USA* 101: 7234−7239

39. Goodrich LV, Strutt D (2011) Principles of planar polarity in animal development. *Development* 138: 1877−1892

40. Antic D, Stubbs JL, Suyama K, Kintner C, Scott MP, Axelrod JD (2010) Planar cell polarity enables posterior localization of nodal cilia and left-right axis determination during mouse and Xenopus embryogenesis. *PLoS ONE* 5: e8999

41. Borovina A, Superina S, Voskas D, Ciruna B (2010) Vangl2 directs the posterior tilting and asymmetric localization of motile primary cilia. *Nat Cell Biol* 12: 407−412

42. Hashimoto M, Shinohara K, Wang J, Ikeuchi S, Yoshiba S, Meno C, Nonaka S, Takada S, Hatta K, Wynshaw-Boris A et al (2010) Planar polarization of node cells determines the rotational axis of node cilia. *Nat Cell Biol* 12: 170−176

43. Song H, Hu J, Chen W, Elliott G, Andre P, Gao B, Yang Y (2010) Planar cell polarity breaks bilateral symmetry by controlling ciliary positioning. *Nature* 466: 378−382

44. Mahaffey JP, Grego-Bessa J, Liem KF Jr, Anderson KV (2013) Cofilin and Vangl2 cooperate in the initiation of planar cell polarity in the mouse embryo. *Development* 140: 1262−1271

45. Norris DP (2012) Cilia, calcium and the basis of left-right asymmetry. *BMC Biol* 10: 102

46. Tanaka Y, Okada Y, Hirokawa N (2005) FGF-induced vesicular release of Sonic hedgehog and retinoic acid in leftward nodal flow is critical for left-right determination. *Nature* 435: 172−177

47. Field S, Riley KL, Grimes DT, Hilton H, Simon M, Powles-Glover N, Siggers P, Bogani D, Greenfield A, Norris DP (2011) Pkd1l1 establishes left-right asymmetry and physically interacts with Pkd2. *Development* 138: 1131−1142

48. Pennekamp P, Karcher C, Fischer A, Schweickert A, Skryabin B, Horst J, Blum M, Dworniczak B (2002) The ion channel polycystin-2 is required for left-right axis determination in mice. *Curr Biol* 12: 938−943

49. Yoshiba S, Shiratori H, Kuo IY, Kawasumi A, Shinohara K, Nonaka S, Asai Y, Sasaki G, Belo JA, Sasaki H et al (2012) Cilia at the node of mouse embryos sense fluid flow for left-right determination via Pkd2. *Science* 338: 226−231

50. Kamura K, Kobayashi D, Uehara Y, Koshida S, Iijima N, Kudo A, Yokoyama T, Takeda H (2011) Pkd1l1 complexes with Pkd2 on motile cilia and functions to establish the left-right axis. *Development* 138: 1121−1129

51. Gardner RL (2010) Normal bias in the direction of fetal rotation depends on blastomere composition during early cleavage in the mouse. *PLoS ONE* 5: e9610

52. Armakolas A, Klar AJ (2007) Left-right dynein motor implicated in selective chromatid segregation in mouse cells. *Science* 315: 100−101

53. Sauer S, Klar AJ (2012) Left-right symmetry breaking in mice by left-right dynein may occur via a biased chromatid segregation mechanism, without directly involving the Nodal gene. *Front Oncol* 2: 166

54. Noel ES, Verhoeven M, Lagendijk AK, Tessadori F, Smith K, Choorapoikayil S, den Hertog J, Bakkers J (2013) A Nodal-independent and tissue-intrinsic mechanism controls heart-looping chirality. *Nat Commun* 4: 2754

55. Feistel K, Blum M (2006) Three types of cilia including a novel 9+4 axoneme on the notochordal plate of the rabbit embryo. *Dev Dyn* 235: 3348−3358

56. Stephen LA, Johnson EJ, Davis GM, McTeir L, Pinkham J, Jaberi N, Davey MG (2014) The chicken left right organizer has nonmotile cilia which are lost in a stage-dependent manner in the talpid ciliopathy. *Genesis* 52: 600−613

57. Cui C, Little CD, Rongish BJ (2009) Rotation of organizer tissue contributes to left-right asymmetry. *Anat Rec* 292: 557−561

58. Gros J, Feistel K, Viebahn C, Blum M, Tabin CJ (2009) Cell movements at Hensen's node establish left/right asymmetric gene expression in the chick. *Science* 324: 941−944

59. Levin M, Thorlin T, Robinson KR, Nogi T, Mercola M (2002) Asymmetries in H^+/K^+-ATPase and cell membrane potentials comprise a very early step in left-right patterning. *Cell* 111: 77−89

60. Adams DS, Robinson KR, Fukumoto T, Yuan S, Albertson RC, Yelick P, Kuo L, McSweeney M, Levin M (2006) Early, H^+-V-ATPase-dependent proton flux is necessary for consistent left-right patterning of non-mammalian vertebrates. *Development* 133: 1657−1671

61. Aw S, Adams DS, Qiu D, Levin M (2008) H,K-ATPase protein localization and Kir4.1 function reveal concordance of three axes during early determination of left-right asymmetry. *Mech Dev* 125: 353–372

62. Shimeld SM, Levin M (2006) Evidence for the regulation of left-right asymmetry in Ciona intestinalis by ion flux. *Dev Dyn* 235: 1543–1553

63. Nishide K, Mugitani M, Kumano G, Nishida H (2012) Neurula rotation determines left-right asymmetry in ascidian tadpole larvae. *Development* 139: 1467–1475

64. Raff RA, Snoke Smith M (2009) Chapter 7. Axis formation and the rapid evolutionary transformation of larval form. *Curr Top Dev Biol* 86: 163–190

65. Molina MD, de Croze N, Haillot E, Lepage T (2013) Nodal: master and commander of the dorsal-ventral and left-right axes in the sea urchin embryo. *Curr Opin Genet Dev* 23: 445–453

66. Duboc V, Rottinger E, Lapraz F, Besnardeau L, 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

67. Frasnelli E, Vallortigara G, Rogers LJ (2012) Left-right asymmetries of behaviour and nervous system in invertebrates. *Neurosci Biobehav Rev* 36: 1273–1291

68. Grande C, Patel NH (2009) Nodal signalling is involved in left-right asymmetry in snails. *Nature* 457: 1007–1011

69. Shimizu K, Iijima M, Setiamarga DH, Sarashina I, Kudoh T, Asami T, Gittenberger E, Endo K (2013) Left-right asymmetric expression of dpp in the mantle of gastropods correlates with asymmetric shell coiling. *Evodevo* 4: 15

70. Kuroda R, Endo B, Abe M, Shimizu M (2009) Chiral blastomere arrangement dictates zygotic left-right asymmetry pathway in snails. *Nature* 462: 790–794

71. Shibazaki Y, Shimizu M, Kuroda R (2004) Body handedness is directed by genetically determined cytoskeletal dynamics in the early embryo. *Curr Biol* 14: 1462–1467

72. Liu MM, Davey JW, Banerjee R, Han J, Yang F, Aboobaker A, Blaxter ML, Davison A (2013) Fine mapping of the pond snail left-right asymmetry (chirality) locus using RAD-Seq and fibre-FISH. *PLoS ONE* 8: e71067

73. Freeman G, Lundelius JW (1982) The developmental genetics of dextrality and sinistrality in the gastropod *Lymnaea peregra*. *Wilhelm Roux's Arc Dev Biol* 191: 69–83

74. Ueshima R, Asami T (2003) Evolution: single-gene speciation by left-right reversal. *Nature* 425: 679

75. Hoso M, Kameda Y, Wu SP, Asami T, Kato M, Hori M (2010) A speciation gene for left-right reversal in snails results in anti-predator adaptation. *Nat Commun* 1: 133

76. Pohl C (2011) Left-right patterning in the *C. elegans* embryo: unique mechanisms and common principles. *Commun Integr Biol* 4: 34–40

77. Wood WB (1998) Handed asymmetry in nematodes. *Semin Cell Dev Biol* 9: 53–60

78. Hutter H, Schnabel R (1995) Establishment of left-right asymmetry in the *Caenorhabditis elegans* embryo: a multistep process involving a series of inductive events. *Development* 121: 3417–3424

79. Hutter H, Schnabel R (1995) Specification of anterior-posterior differences within the AB lineage in the *C. elegans* embryo: a polarising induction. *Development* 121: 1559–1568

80. Pohl C, Bao Z (2010) Chiral forces organize left-right patterning in *C. elegans* by uncoupling midline and anteroposterior axis. *Dev Cell* 19: 402–412

81. Bergmann DC, Lee M, Robertson B, Tsou MF, Rose LS, Wood WB (2003) Embryonic handedness choice in *C. elegans* involves the Galpha protein GPA-16. *Development* 130: 5731–5740

82. Johnston CA, Afshar K, Snyder JT, Tall GG, Gonczy P, Siderovski DP, Willard FS (2008) Structural determinants underlying the temperature-sensitive nature of a Galpha mutant in asymmetric cell division of *Caenorhabditis elegans*. *J Biol Chem* 283: 21550–21558

83. Cabello J, Neukomm LJ, Gunesdogan U, Burkart K, Charette SJ, Lochnit G, Hengartner MO, Schnabel R (2010) The Wnt pathway controls cell death engulfment, spindle orientation, and migration through CED-10/Rac. *PLoS Biol* 8: e1000297

84. Bischoff M, Schnabel R (2006) A posterior centre establishes and maintains polarity of the *Caenorhabditis elegans* embryo by a Wnt-dependent relay mechanism. *PLoS Biol* 4: e396

85. Hermann GJ, Leung B, Priess JR (2000) Left-right asymmetry in *C. elegans* intestine organogenesis involves a LIN-12/Notch signaling pathway. *Development* 127: 3429–3440

86. Wood WB (1991) Evidence from reversal of handedness in *C. elegans* embryos for early cell interactions determining cell fates. *Nature* 349: 536–538

87. Hobert O, Johnston RJ Jr, Chang S (2002) Left-right asymmetry in the nervous system: the *Caenorhabditis elegans* model. *Nat Rev Neurosci* 3: 629–640

88. Troemel ER, Sagasti A, Bargmann CI (1999) Lateral signaling mediated by axon contact and calcium entry regulates asymmetric odorant receptor expression in *C. elegans*. *Cell* 99: 387–398

89. Poole RJ, Hobert O (2006) Early embryonic programming of neuronal left/right asymmetry in *C. elegans*. *Curr Biol* 16: 2279–2292

90. Bertrand V, Bisso P, Poole RJ, Hobert O (2011) Notch-dependent induction of left/right asymmetry in *C. elegans* interneurons and motoneurons. *Curr Biol* 21: 1225–1231

91. Cochella L, Hobert O (2012) Embryonic priming of a miRNA locus predetermines postmitotic neuronal left/right asymmetry in *C. elegans*. *Cell* 151: 1229–1242

92. Zhang F, O'Meara MM, Hobert O (2011) A left/right asymmetric neuronal differentiation program is controlled by the *Caenorhabditis elegans* lsy-27 zinc-finger transcription factor. *Genetics* 188: 753–759

93. Coutelis JB, Geminard C, Speder P, Suzanne M, Petzoldt AG, Noselli S (2013) Drosophila left/right asymmetry establishment is controlled by the Hox gene abdominal-B. *Dev Cell* 24: 89–97

94. Speder P, Adam G, Noselli S (2006) Type ID unconventional myosin controls left-right asymmetry in *Drosophila*. *Nature* 440: 803–807

95. Geminard C, Gonzalez-Morales N, Coutelis JB, Noselli S (2014) The myosin ID pathway and left-right asymmetry in Drosophila. *Genesis* 52: 471–480

96. Coutelis JB, Petzoldt AG, Speder P, Suzanne M, Noselli S (2008) Left-right asymmetry in *Drosophila*. *Semin Cell Dev Biol* 19: 252–262

97. Hozumi S, Maeda R, Taniguchi K, Kanai M, Shirakabe S, Sasamura T, Spéder P, Noselli S, Aigaki T, Murakami R *et al* (2006) An unconventional myosin in *Drosophila* reverses the default handedness in visceral organs. *Nature* 440: 798–802

98. Hozumi S, Maeda R, Taniguchi-Kanai M, Okumura T, Taniguchi K, Kawakatsu Y, Nakazawa N, Hatori R, Matsuno K (2008) Head region of unconventional myosin I family members is responsible for the organ-specificity of their roles in left-right polarity in *Drosophila*. *Dev Dyn* 237: 3528–3537

99. Suzanne M, Petzoldt AG, Speder P, Coutelis JB, Steller H, Noselli S (2010) Coupling of apoptosis and L/R patterning controls stepwise organ looping. *Curr Biol* 20: 1773–1778

100. Maeda RK, Karch F (2009) The bithorax complex of *Drosophila* an exceptional Hox cluster. *Curr Top Dev Biol* 88: 1–33

101. Speder P, Noselli S (2007) Left-right asymmetry: class I myosins show the direction. *Curr Opin Cell Biol* 19: 82–87

102. Krendel M, Mooseker MS (2005) Myosins: tails (and heads) of functional diversity. *Physiology (Bethesda)* 20: 239–251

103. Nambiar R, McConnell RE, Tyska MJ (2010) Myosin motor function: the ins and outs of actin-based membrane protrusions. *Cell Mol Life Sci* 67: 1239–1254

104. Barylko B, Binns DD, Albanesi JP (2000) Regulation of the enzymatic and motor activities of myosin I. *Biochim Biophys Acta* 1496: 23–35

105. Greenberg MJ, Ostap EM (2013) Regulation and control of myosin-I by the motor and light chain-binding domains. *Trends Cell Biol* 23: 81–89

106. Hokanson DE, Laakso JM, Lin T, Sept D, Ostap EM (2006) Myo1c binds phosphoinositides through a putative pleckstrin homology domain. *Mol Biol Cell* 17: 4856–4865

107. Hokanson DE, Ostap EM (2006) Myo1c binds tightly and specifically to phosphatidylinositol 4,5-bisphosphate and inositol 1,4,5-trisphosphate. *Proc Natl Acad Sci USA* 103: 3118–3123

108. Taniguchi K, Maeda R, Ando T, Okumura T, Nakazawa N, Hatori R, Nakamura M, Hozumi S, Fujiwara H, Matsuno K (2011) Chirality in planar cell shape contributes to left-right asymmetric epithelial morphogenesis. *Science* 333: 339–341

109. Petzoldt AG, Coutelis JB, Geminard C, Speder P, Suzanne M, Cerezo D, Noselli S (2012) DE-Cadherin regulates unconventional Myosin ID and Myosin IC in Drosophila left-right asymmetry establishment. *Development* 139: 1874–1884

110. Chen TH, Hsu JJ, Zhao X, Guo C, Wong MN, Huang Y, Li Z, Garfinkel A, Ho CM, Tintut Y *et al* (2012) Left-right symmetry breaking in tissue morphogenesis via cytoskeletal mechanics. *Circ Res* 110: 551–559

111. Wan LQ, Ronaldson K, Park M, Taylor G, Zhang Y, Gimble JM, Vunjak-Novakovic G (2011) Micropatterned mammalian cells exhibit phenotype-specific left-right asymmetry. *Proc Natl Acad Sci USA* 108: 12295–12300

112. Wan LQ, Vunjak-Novakovic G (2011) Micropatterning chiral morphogenesis. *Commun Integr Biol* 4: 745–748

113. Xu J, Van Keymeulen A, Wakida NM, Carlton P, Berns MW, Bourne HR (2007) Polarity reveals intrinsic cell chirality. *Proc Natl Acad Sci USA* 104: 9296–9300

114. Pyrpassopoulos S, Feeser EA, Mazerik JN, Tyska MJ, Ostap EM (2012) Membrane-bound myo1c powers asymmetric motility of actin filaments. *Curr Biol* 22: 1688–1692

115. Kim SV, Flavell RA (2008) Myosin I: from yeast to human. *Cell Mol Life Sci* 65: 2128–2137

116. Richards TA, Cavalier-Smith T (2005) Myosin domain evolution and the primary divergence of eukaryotes. *Nature* 436: 1113–1118

117. Berg JS, Powell BC, Cheney RE (2001) A millennial myosin census. *Mol Biol Cell* 12: 780–794

118. Tzolovsky G, Millo H, Pathirana S, Wood T, Bownes M (2002) Identification and phylogenetic analysis of *Drosophila melanogaster* myosins. *Mol Biol Evol* 19: 1041–1052