Left-Right Asymmetry
in Animal Embryogenesis

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Introduction

The geometrical invariance known as symmetry is a striking feature of developmental morphology during embryogenesis. There are several types, such as translational symmetry (repeated units such millipede segments), and reflectional symmetry (two or more sections of an organism looking the same to some level of detail on either side of a symmetry line). Animal body-plans occur in a wide variety of symmetries (see Figure 1). Vertebrates have a generally bilaterally-symmetrical body-plan, but this symmetry is broken further into a pseudo-symmetry by the consistently asymmetric placement of various internal organs such as the heart, liver, spleen, and gut, or an asymmetric development of paired organs (such as brain hemispheres or lungs).

Symmetries are repeatedly broken during development. For example, the radial symmetry of the early chick blastoderm (see Fig. 2) is broken into a bilateral symmetry by the appearance of Köhler's sickle and then the primitive streak. This is further broken into a definitive pseudo-symmetry by the right-sided looping of the heart tube. In contrast, the sea-urchin develops from a bilaterally-symmetric larva into an adult with a five-fold radial symmetry.

Arguably, the most interesting asymmetry in vertebrate development is that along the left-right (LR) axis. I limit this discussion to include only invariant (i.e., consistent among all normal individuals of a given type) differences between the left and right sides of an animal's morphology. This specifically exclude pseudo-random characteristics such as animal coat colors, and minor stochastic deviations due to developmental noise.

The LR axis itself follows automatically from the definition of the AP and DV axes, as it is perpendicular to both; however, consistently imposed asymmetry across it is fundamentally different from patterning along the other two axes. Firstly, while the AP and DV axes can be set by exogenous cues such as gravity, or sperm entry point, there is no independent way to pick out the left (or right) direction, since no obvious macroscopic aspect of nature differentiates left from right. Secondly, all normal members of a given species are asymmetrical in the same direction. However, animals with complete mirror reversal of internal organs can arise (*situs inversus*) and are otherwise phenotypically unimpaired. Thus, while it is possible to come up with plausible evolutionary reasons for why organisms might be asymmetric in the first place (optimal packing of viscera, etc.), there is no obvious reason for why they should all be asymmetric in the same direction. It is, after all, much easier to imagine a developmental mechanism for generating asymmetry (such as positive-feedback and amplification of stochastic biochemical differences) than for biasing it to a given direction. The left-right axis thus presents several unique and deeply interesting theoretical issues.

A priori, one can imagine several ways to generate consistent LR asymmetry in an embryo. One way would be to orient the embryo within the mother organism (Fig. 3A); thus the asymmetry would derive directly from LR-asymmetric influences applied by the already asymmetric maternal organism. While this might be plausible in mammals, the consistent asymmetry in free-developing organisms argues against the necessity for this kind of mechanism. Another possibility is the generation of prepattern in the egg (Fig. 3B). Thus, if the oocyte was asymmetrically loaded with determinants by the maternal ovary, these determinants could then go on to elaborate the LR asymmetry during later development. However, the regulative nature of development argues against this mechanism; for example, blastomeres of mouse embryos can be scrambled, split, or added to (i.e., 2 embryos made to aggregate together) and still result in phenotypically normal organisms. Another interesting mechanism makes use of a fundamental force of physics to orient the LR axis relative to the other two axes (Fig. 3C). Huxley and deBeer [1] proposed that LR asymmetry was oriented during embryonic development by an electric current running down the length of the notochord, which would generate a magnetic field pointing R or L, if measured at the dorsal or ventral sides. There is, however, no good evidence for such a mechanism. The most plausible mechanism for generating left-right asymmetry makes use of molecular chirality (Fig. 3D). If a chiral molecule (shown as a 2-dimensional "F", after Brown and Wolpert, 1990 [2]) has a

directional activity (such as transport of subcellular components, or nucleation of directed microtubules) and is oriented within the cell relative to the other 2 axes, it can generate a consistent asymmetry.

Whatever mechanism initially differentiates L from R represents the first basic step of LR asymmetry (Fig. 4). The next step consists of elaborating this information into multi-cellular fields of asymmetric gene expression. As will be discussed below, there has been identified a cascade of genes which are expressed only on the left or the right side of the body, and regulate (turn on or off) each other's expression. In the final step, tissues which are forming asymmetric organs such as hearts and stomachs take cues from asymmetrically expressed genes and undergo sided morphogenesis. Several labs have made significant progress in working out the details of the regulatory interactions between the known asymmetric genes, and elucidating mechanisms by which organ primordia interact with such genes; thus, we are well on our way to understanding stages 2 and 3. The most fascinating questions however concern stage 1, and are still almost completely open. These will be touched upon at the end of this paper.

Besides the intrinsic interest to those working on fundamental morphogenetic mechanisms, LR asymmetry is also relevant to medical considerations of several fairly common human birth defects: syndromes as Kartagener's and Ivemark's [3], dextrocardia, *situs inversus* (a complete mirror-image reversal of the sidedness of asymmetrically positioned organs and asymmetric paired organs), heterotaxia (where each organ makes an independent decision as to its *situs*), and right or left isomerism (where the organism is completely symmetrical, for example, polysplenia or asplenia). Of these, only the complete (and rare) *situs inversus* is not associated with physiological difficulties. The rest, especially heterotaxia, often result in serious health problems for the patient.

Pre-molecular data

While molecular mechanisms underlying antero-posterior and dorso-ventral asymmetry have been studied in detail, the mechanistic basis for LR asymmetry was, until recently, completely unknown. The bilateral body plan is thought to have originated with the eumetazoa. The LR axis is specified after the anterior-posterior (AP) and dorso-ventral (DV) axes, and is determined with respect to them [4,5]. Currently, several morphological markers of LR asymmetry are apparent in vertebrates: heart, direction of embryo rotation, gut, liver, lungs, etc. The organs possessing asymmetries, as well as the direction of their asymmetry, are evolutionarily well conserved. The heart is asymmetrically located in the mollusks [6]; the *situs* of the stomach and the liver [7] is the same among fish, reptiles, birds, and mammals.

Neville [8] presents an extensive and fascinating survey of various animal asymmetries. Besides the above-mentioned internal organs, beetles consistently fold one wing under the other, many crustaceans have specialized right and left fore-limbs, some flatfish consistently settle on and undergo eye migration to one side, and there is even a species of parasite which lives only on one side of host shrimp. Meanwhile, there has been little information shedding light on the mechanisms determining the sidedness of the asymmetries. Selection for LR asymmetries in *Drosophila*, in hopes of generating a genetically-tractable mutant, failed [9].

Several experiments have shed light on the timing of LR asymmetry specification. Chick heart sidedness has been experimentally demonstrated to be determined during gastrulation [10]; studies on LR inversions induced by drugs likewise suggest that in mammals, a critical period in LR biasing occurs before late gastrulation [11]. Thus it is clear that decisions fundamental to LR asymmetry are made long before any overt signs of morphological asymmetry, and long before the morphogenesis of asymmetric organs.

Several kinds of mollusks undergo spiral cleavage and secrete an exoskeleton shaped like a conical spiral. In 3D space, such spirals can have two possible variants: a left-handed and a right-

handed helix (which are otherwise identical). Each particular species of snail has invariant (consistent) chirality, but there are species which utilize each type of coiling. Murray and Clarke [12] found that the direction of coiling of *P. suturalis* is maternally inherited and sinistrality is dominant to dextrality. Freeman and Lundelius [13], studying a different species, found that dextrality is dominant; interestingly, the dextral gene apparently functions via a cytoplasmic product since it is possible to transfer (by micro-pipette) cytoplasm from the dextral variant of the snail into the sinistral variety, and rescue the dextral coiling phenotype. The biochemical nature of this activity has not yet been identified.

There is a variety of drugs which cause defects in a LR-asymmetric manner or randomize asymmetry (Table 1). These form a basically unrelated group, which includes even such simple substances as cadmium. The drugs which cause worse limb defects on one side were suggested [14] to be due to a differential blood supply to the two limbs (due to asymmetry in blood vessels exiting the heart). This is made somewhat unlikely by the fact that cadmium causes opposite-sided defects in rats and mice [15,16], while cardiac anatomy and relative vessel size of both species are extremely similar. This suggests a fundamental difference between left and right limbs. The pharmacology of these drugs has not yet suggested anything about the normal mechanisms of LR patterning, except that an adrenergic pathway may be involved [11].

Several mammalian mutants are known which display either defects in basic LR patterning or phenotypes which differentially affect the left or right sides of the body (Table 2). For example, *iv* [17] results in racemic offspring (50% being phenotypically *situs inversus*), while *inv* [18] mice have 100% of the offspring showing mirror image inversions of the internal organs (although in the context of other heterotaxia-like phenotypes, [19]). Mutants such as *legless* [20] exhibit limb phenotypes which are more pronounced on one side of the body. In crosses with *iv*, the side affected is shown to reverse with the organ *situs*.

Regulatory cascade of asymmetric gene expression

A number of asymmetrically-expressed genes have now been described (see Table 3). These include a variety of signaling molecules and transcription factors. Figure 5 illustrates the expression pattern of three such genes as assayed by *in situ* hybridization with riboprobes to the relevant genes: *Sonic Hedgehog* (Shh), *Nodal*, and *PTC* [21]. Beginning with the studies of Levin *et al*. [21], it was discovered that *Shh* is expressed only on the left side of Hensen's node in the gastrulating chick embryo (Figure 5A). Shortly thereafter, *Nodal* and *PTC* are expressed also on the left side.

Once a set of asymmetrically-expressed genes was identified, their location and relative timing of expression suggested a possible pathway of sequential inductions and repressions. Using artificial retroviruses bearing the gene of interest and protein-coated beads, a pathway was constructed. For example, it was found that misexpressing the normally left-sided gene *Shh* on the right side caused the ectopic right-sided expression of *Nodal*, which is normally also confined to the left side. This cascade (summarized in Figure 6) begins when *activin* β*B* becomes expressed on the right side of Hensen's node (st. 3). This soon induces the expression of *cAct-RIIa* in the right side, and shuts off the right-side expression of *Shh* (which was previously expressed throughout the node). Soon thereafter, *Shh* (which at that point is expressed only on the left side of the node and in the notochord) induces *nodal* in a small domain of cells adjacent to the left side of the node. This is soon followed by a much larger domain in the lateral plate mesoderm.

Most importantly, the early asymmetrically-expressed genes are not merely markers of inherent laterality, but play an active role in LR patterning. Misexpression of *activin* or *Shh* (which result in missing or bilateral *nodal* expression respectively) specifically randomize heart *situs* in the

chick [21]. Moreover, *nodal*, which is in direct contact with cardiac precursor cells, can reverse heart *situs* or cause symmetric hearts [22]. Thus, though there is no consensus on what causes cardiac looping in the first place, it is plausible that *nodal* is instructing heart looping by providing an asymmetric signal to one side of the cardiac primordia, and affecting the proliferation, migration, or cytoskeletal organization of cardiac precursors. The fact that morphologically normal hearts form in the absence of *Shh* and *nodal* expression (albeit with randomization of heart *situs*) indicates that the genes in this cascade are neither responsible for inducing heart formation nor for instructing its morphogenesis. Rather, they seem to provide a pivotal influence determining the handedness of the heart. Interestingly, the other organs besides the heart likewise take their cues from this genetic cascade [22].

Laterality Disturbances in Twins and the Midline Barrier

The identification and characterization of several players in LR patterning has enabled models explaining the finding that conjoined twins of armadillo [23], fish [24], frog [25], and man [3,26], often exhibit alterations of *situs* in one of the twins. As early as 1919, Spemann and Falkenberg [27] reported that producing conjoined twins by tying a hair between the two blastomeres of amphibian eggs results in *situs inversus* in one of the twins. Levin *et al*. (1995) suggest that an explanation for the association of laterality defects and twinning might be found in consideration of interactions between signaling molecules in two closely aligned primitive streaks.

Several types of human conjoined twins are shown in Fig. 7A. Since the identified molecules participating in the LR cascade include many diffusible signaling molecules, it is reasonable to suppose that in cases of side-by-side twins (as in parapagus twins) the asymmetrically-expressed genes on one side of one twin might affect the development of the opposite side of the adjacent twin. Indeed, it was found that unlike the other types of twins, human twins connected side by side have significantly much higher incidences of laterality disturbances [28]. Levin *et al*. (1996)[28] examined spontaneous conjoined chicken twins of the parapagus type. One such example is shown in Figure 7B; note that the left twin has bilateral expression of the gene *Nodal*, which should normally only be expressed on the left side (as it is, in the right twin).

These findings have suggested the following model which explains laterality defects in conjoined defects as being due to interactions of laterality-determining factors among juxtaposed streaks. The primary (head-tail) axis of embryos arise from the primitive streak. In conjoined embryos of the type shown in Figure 7B, two streaks began at separate points (Figure 7C) but get closer as they extend, and eventually fuse at the cranial end (Figure 7D). At that point, the left-sided expression of *Shh* can influence the nearby right side of the Hensen's node of the left twin, and induce ectopic *Nodal* expression on its right side [21]. This is exactly what is observed in spontaneous head-to-head chick and human twins (Fig. 7B). This cascade is schematized in Figure 7E.

Interestingly, the model of laterality defects based on cross-embryo signaling cannot be the whole story. Non-conjoined monozygotic twins, while not exhibiting the kinds of visceral laterality defects that occur in conjoined twins, do manifest many subtler kinds of mirror-image asymmetry. Pairs of such twins have been noted to present mirror asymmetries in hand preference, hair whorl direction, tooth patterns, unilateral eye and ear defects, and even tumor locations and un-descended testicles [29-36]. Most healthy, non-conjoined twins presumably result from separation of cleavage, morula, or early blastocyst stage embryos [37]: it is much easier to imagine the splitting of a 2-cell embryo rather than a complex structure such as the egg cylinder (twinning at that late stage would seem likely to yield conjoined or incompletely-patterned twins). Thus, some chiral information may be present in the very early mammalian embryo, manifesting itself in hair whorls etc. if the cells are separated at an early stage. In contrast, the asymmetry of the major body organs seems to be

unspecified at those stages, and is developed correctly for both monozygotic twins. This may be related to the fact that heterotaxic reversals in hair whorls and tooth patterns would not be expected to be disadvantageous, while discordant *situs* for internal organs clearly is subject to negative evolutionary pressure.

Remaining Puzzles

The studies of the genetic cascades involved in LR axis orientation are a fundamental advance; but however far backwards the gene cascade is followed, one must ask: whichever gene is asymmetrically expressed first, what is the cause of its asymmetry? The most likely primal source of embryonic left-right asymmetry is the chiral nature of some molecule or subcellular component (such as centrioles). Much of the thinking regarding coordination of the three cardinal body axes has been influenced by Brown and Wolpert who proposed the involvement of a chiral molecule, termed the "F" molecule, that would recognize the polarity of two fixed axes to orient the third [2]. An attractive possibility is that microtubules are fundamentally involved [38]. Microtubules may be involved as part of the input or the output of the calculation which combines LR, AP, and DV information. In one model [2,39], microtubules may be oriented with respect to either AP or DV, and permit binding of a chiral "F" molecule. Additional input from the remaining unaligned axis (DV or AP) would fix the direction of the "F" molecule and thus orient the LR axis. Alternatively, in the output model [40], a chiral "F" molecule which is oriented with respect to the AP and DV axes could initiate microtubule nucleation along the LR axis (minus end to the left, for example), allowing the easy unidirectional transport of LR determinants by proteins such as dynein. These determinants can then go on to induce one-sided gene expression. Interestingly, the microtubule motor protein dynein has been shown to be mutated in the mouse mutant *iv* [41].

An additional mechanism for integrating DV and LR information, as well as for asserting cellular LR differences across multi-cellular fields of cells involves gap junctions [40]. Gap junctions between cells allow the passage of small signaling and have been recently shown to be critically involved in early generation of left-right asymmetry [42]. By regulating the flow of small LR morphogens, differential dorso-ventral patterns of GJC can result in a left-right asymmetric distribution of such molecules on an embryo-wide scale.

Even more elusive are the questions of why and how left-right asymmetry arose evolutionarily. It is unclear whether asymmetry (or more likely, chirality) is basic to the animal body-plan, and the seeming outward symmetry of most animals a later modification, or whether asymmetry is the later tweak that is imposed on a basically symmetrical system [43]. Likewise, it is entirely unclear why consistent asymmetry is so prevalent. One could argue that some asymmetry is necessary in organs such as the gut and heart, for physiological reasons[44]. This is consistent with the observation that the degree of left-right symmetry can be used to gauge the genetic and developmental "robustness" of an animal, both by ecologists [45] and by other animals (as in the role symmetry plays in the human judgment of facial beauty and in non-human mate choice [46]).

Mechanisms for generating asymmetry between two sides are also easy to imagine, given gene networks and magnification of small stochastic differences (as in the Notch-Delta system [47]). Given the ease of generating such random asymmetry, and given that animals with full *situs inversus* appear phenotypically unimpaired, why are not all animal populations a racemic mixture of opposite enantiomers in a 1:1 ratio? The ubiquity of consistently biased, not simply asymmetric, species suggests that either the biasing component is an extremely old vestige of our evolution, or that for some unknown reason it is not possible to produce offspring with a pure 50:50 incidence of *situs inversus totalis* and *situs solitus*. This impossibility is consistent with the observation that the *iv* mouse, usually thought of as instantiating this possibility, actually has significant incidence of heterotaxia, and is thus phenotypically impaired [48]. Interestingly, the sinistral forms of certain

chiral snail shells are seen, upon close inspection, to also imply consequences for shell form aside from chirality [49].

Another interesting issue concerns the degree of linkage of visceral and neurological asymmetry. Brain lateralization and hand preference are popular examples of LR asymmetry [50]. Amazingly, patients with *situs inversus* exhibit the same low incidence of left-handedness as is found in the general population [51,52]. The fact that developmental processes can be perturbed in such a way as to fully reverse morphological asymmetry of the viscera but leave brain asymmetry in its normal bias suggests either that the mechanisms controlling neurological asymmetry comprise a completely separate pathway from those controlling body *situs*, or that they are linked, but that mutations giving rise to human laterality defects have so far occurred at points downstream of the divergence of the two pathways. Given that most manipulations studied to date involve all visceral organs, the latter possibility would imply that neurological asymmetry is calculated and set apart from body *situs* quite early in development.

The issue of original chirality (i.e., why living organisms contain L-amino acids and D-sugars) is also a very interesting one, and is bound up fundamentally with the origin of life. Perhaps, whatever type of isomer happened to have formed first biased the rest of evolution towards that type by competition [53]. The chirality of the first one could have been determined by chance, or by exogenous factors such as the Coriolis force, light [54], or even the geomagnetic field. Interestingly, the GMF seems to have a relationship with LR chirality [55]. The geological fossil record shows a clear correlation between flipping of the GMF polarity and reversals of the chirality of several types of mollusks such as *Globorotalia menardi* [56,57].

Alternatively, there may be a fundamental reason for why biological forms prefer one type of molecule over its enantiomer. For example, when racemic mixtures of the amino acids alanine, tryptophan, and tyrosine in alkaline solution are subjected to decomposition by radio-active decay of strontium-90, the D-isomers are destroyed more quickly than the L-isomer[58]. There are also arguments [59] based on weak neutral currents which show that the terrestrially dominant L-amino acids will predominate in a period of on the order of 15,000 years. Thus, radio-active decay could plausibly have biased enantiomer choice in the pre-biotic environment. Likewise, the energy of the right-handed α-helix of poly-L-alanine is a few tenths of a kilocalorie per mole per residue lower than that of the left-handed helix, implying that over some length, the right-handed forms will be more stable; both asymmetries are presumably consequences of the non-conservation of parity in sub-atomic weak nuclear interactions [60]. Thus, embryonic morphogenesis promises many fascinating insights on the linkage between subatomic chirality and large-scale asymmetry in animal bodyplans.

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Table 1. *Drugs with asymmetric effects*

Substance	Type	Species	Phenotype	Ref.
Cadmium	Element	Rat	Left limb deformities	15
Cadmium	Element	Mouse	Right limb deformities	16
Acetazolamide	Carbonic anhydrase inhibitor	Rat	Right limb deformities	61
MNNG	Alkylating agent	Mouse	Left ecodactyly	62
Acetoxymethylmethylnitrosamine	Alkylating agent	Mouse	Left limb deformities	63
Xyloside	Proteoglycan synthesis inhibitor	Frog	No cardiac looping	64
Nitrous oxide	Anesthetic	Rat	Situs inversus viscerum	65
Retinoic acid	Teratogen	Hamster	Situs inversus	66
Phenylephrine	Adrenergic agonist	Rat	Situs inversus viscerum	11
Methoxamine	Adrenergic agonist	Rat	Situs inversus and heterotaxia	67
Staurosporine	PKC inhibitor	Rat	Situs inversus	68
Lidocaine	Local anesthetic	Rat	Situs inversus	69
Nitrofurazone	Antimicrobial agent	Rat	Right-sided hypoplasia	70
RGD polypeptides	Blocks ECM attachment	Frog	Situs inversus viscerum	71

Table 2. *Mutants with LR asymmetry phenotypes*

Name	Species	Phenotype	Reference
Mgat-1$^{-/-}$	Mouse	Randomized turning and heart	72
Ft	Mouse	Randomized turning, normal heart	73
Inv	Mouse	100% *situs inversus*	18
Iv	Mouse	50% *situs inversus*	17
Legless	Mouse	Right limb defects	74
Heterotaxia	Human	Independent situs of internal organs	75
Dh	Mouse	*Situs inversus*	76
Hyd	Rat	*Situs inversus*	77
Py	Mouse	Right limb defects	78
Roller	C. elegans	Left or right twisted helical morphology	79
Glp-1$^{(e2072)}$	C. elegans	Almost true isomerism	80

Table 3. *Genes asymmetrically expressed in embryos*

Gene	Species	Product/Role	Side	Ref
lefty	mouse	TGF-β-family signaling molecule	Left	81
Activin βB	chick	TGF-β-family signaling molecule	Right	22
cAct-RIIa	chick	Activin receptor	Right	21
Shh	chick	Signaling molecule	Left	21
cSnR	chick	Zinc finger protein	Right	82
HNF3-β	chick	Winged-helix transcription factor	Left	21
nodal	chick, mouse, frog	TGF-β-family signaling molecule	Left	21,83,84
cWnt-8C	chick	wnt-family signaling molecule	Right	*
cPTC	chick	Receptor	Left	*
HGF	chick	kringle signaling molecule	Left	85
Hrlim	ascidian	LIM-family signaling molecule	Right	86
Ptx	Chick, mouse, frog	Hox gene	Left	87
follistatin	chick	Activin binding protein	Right	88

Figure Legends

Figure 1: Symmetry types in the animal kingdom.

The bodyplans of animals exhibit a variety of symmetry types, including spherical, radial, and bilateral. Onto these basic symmetries is often superimposed a subtle asymmetry, resulting in chiral or pseudo-bilateral forms.

Figure 2: Symmetry breakage during chick development.

The early chick embryo is a flat disk of cells, two cell layers thick (A). It is morphologically radially symmetrical at this stage. During gastrulation (B,C) the primitive streak appears at some circumferential point and reduces the symmetry to a bilateral one. Finally, the asymmetric looping of the heart tube reduces the symmetry further to a pseudobilateral asymmetry. Straight lines indicate axes of symmetry.

Figure 3: Possible mechanisms for establishing left-right asymmetry.

(A) Organisms which develop in contact with some part of the maternal organism (such as mammals) can derive LR cues (shown in green) from their environment. (B) Oocytes can be asymmetrically patterned by the maternal ovary cells. (C) Electric currents flowing along the AP axis generate magnetic fields which point L or R. (D) A chiral molecule which is tethered with respect to the AP and DV axes can have a directional activity (such as nucleating microtubule assembly or providing transport) in one direction along the LR axis (shown by arrowheads). A = Anterior; P = Posterior; L = Left; R = Right; the DorsoVentral axis is in the plane of the page.

Figure 4: Three fundamental steps of left-right patterning.

The first step is the differentiation of L from R on a single cell or molecular level, and the orientation of the LR axis with the DV and AP axes. The second step is represented by the imposition of single-cell LR information on embryo-wide global cell fields, resulting in asymmetric gene expression. The final step is accomplished when the asymmetric organs such as the heart, stomach, liver, etc. read cues established by the asymmetric gene cascade and undergo chiral morphogenesis.

Figure 5: Sample asymmetrically-expressed genes.

(A) *Shh* is expressed on the left side of Hensen's node (the top of the primitive streak) and throughout the notochord emerging from the top of Hensen's node. (B) *Nodal* is expressed in a smaller and a larger domain, both on the left side of the embryonic midline. *Nodal* is indicated in dark blue stain; pre-cardiac cells (symmetrically located) are indicated in magenta stain. (C) *PTC* is expressed on the left side of the late Hensen's node. Black arrows indicate expression, white arrows indicate lack of expression on the contralateral side.

Figure 6: Part of the pathway of asymmetrically expressed gene.

Shh is initially expressed on both sides of Hensen's node. When *activin* becomes expressed on the right side, it induces *cAct-RIIa* and represses *Shh* there, leaving only left-sided *Shh* expression. The left-sided *Shh* expression goes on to induce expression of *nodal* on the left side, which subsequently is transduced to the asymmetric morphogenesis of the viscera.

Figure 7: Conjoined twins and laterality defects.

(A) Several types of spontaneous conjoined twinning events occur in human births. Parapagus and Thoracopagus are the only types associated with laterality defects. (B) Spontaneous chicken twins joined at the head exhibit alterations in the normal asymmetry of genes such as *Nodal*; it is seen that the left twin exhibits bilateral *Nodal* expression while the right twin is normal (arrows indicate expression). These observations suggest a model: twins formed by primary axes (primitive streaks) growing together (C) exhibit laterality defects because, for example, (D) the right twin's *Shh* expression in the left half of Hensen's node induces not only its own *Nodal* expression but also ectopic *Nodal* expression in the right side of the left twin (black arrow). This series of events is schematized in panel (E). Black arrows represent inductions.

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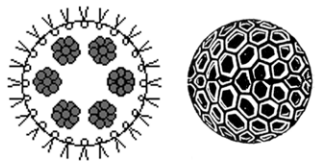
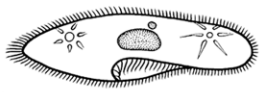
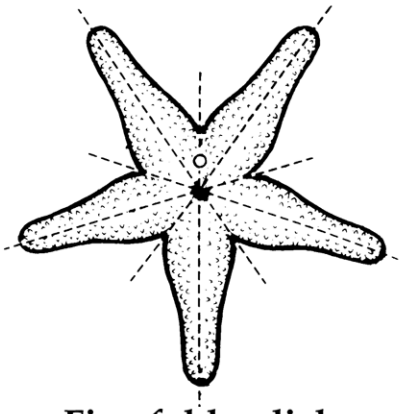
Figure 1: symmetry types in animal morphologies



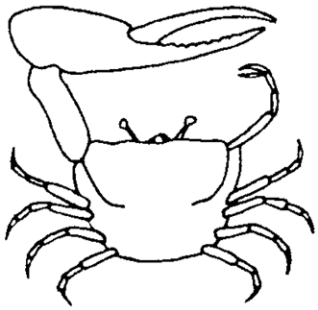
Spherical



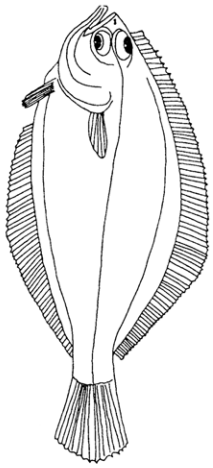
Chiral



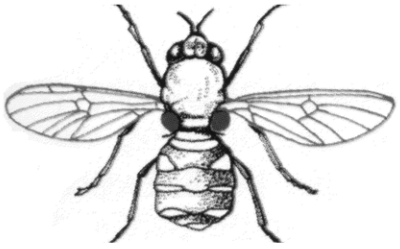
Five-fold radial



Pseudo-bilateral

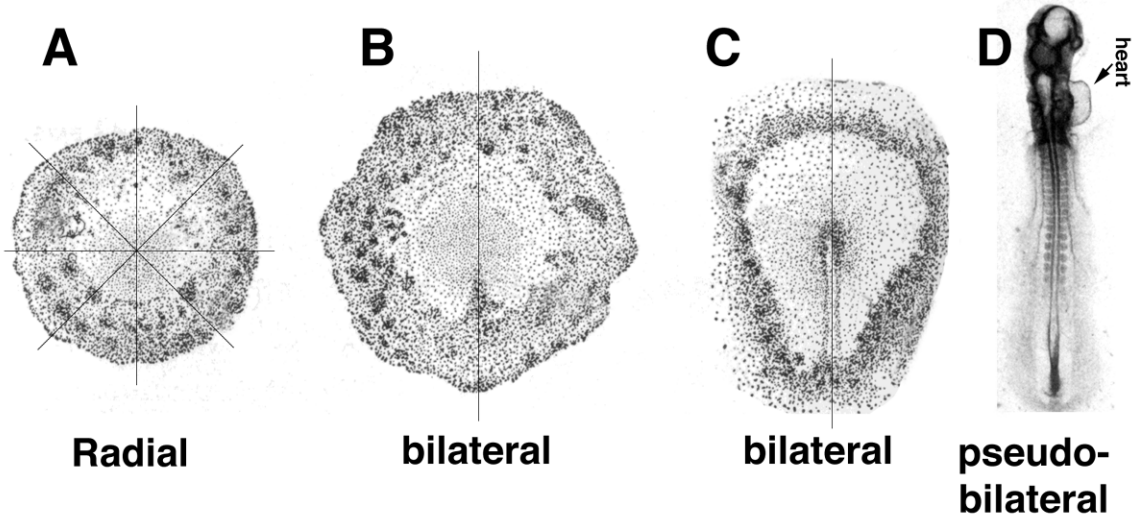


radial



Bilateral

Figure 2: symmetry breaking in embryogenesis



Radial

bilateral

bilateral

pseudo-bilateral

Figure 3: mechanisms of initial LR asymmetry

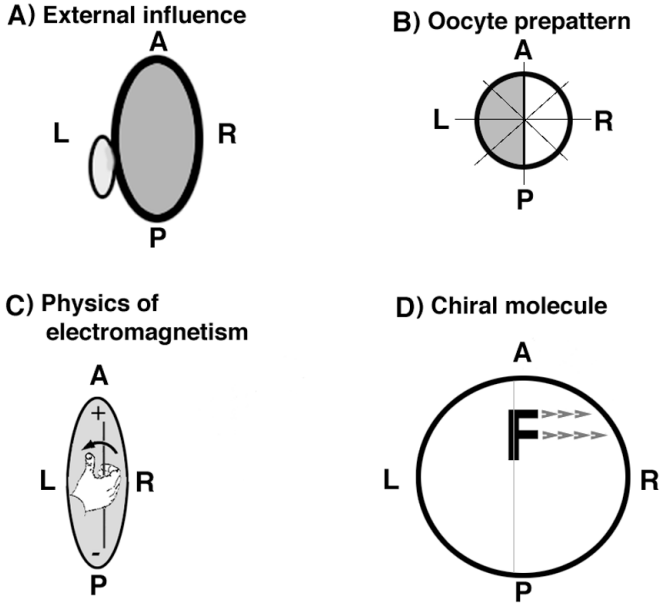


Figure 4: stages of LR patterning

Differentiate L from R by orienting 3rd axis with respect to AP & DV

(F-shaped molecule, dynein, cytoskeleton, cleavage planes, chirality, etc.)

↓

Elaborate this information into multicellular fields - asymmetric gene expression

(pathway of asymmetrically-expressed genes)

↓

Orient and shape morphogenesis of individual organs (heart, gut, lungs, gall bladder, etc.)

(differential cell proliferation, migration, and cytoskeletal organization)

Figure 5: sample asymmetric genes

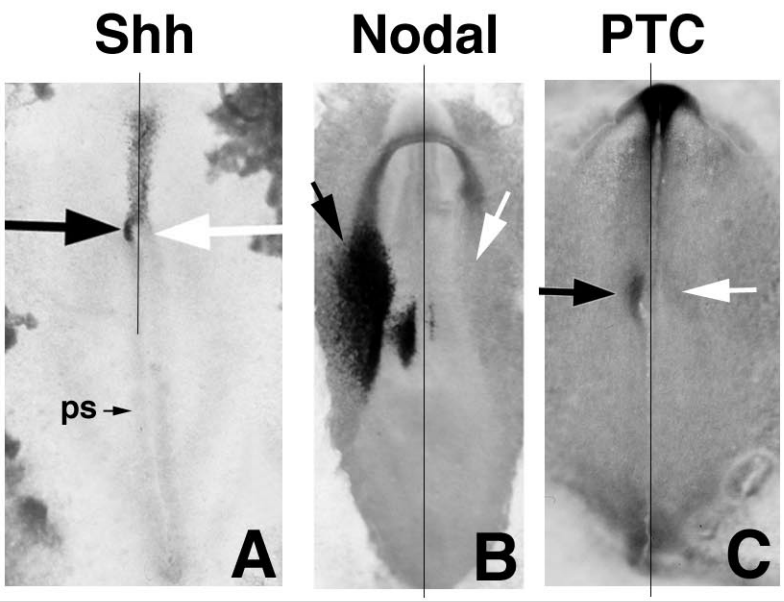


Figure 6: cascade of LR genes

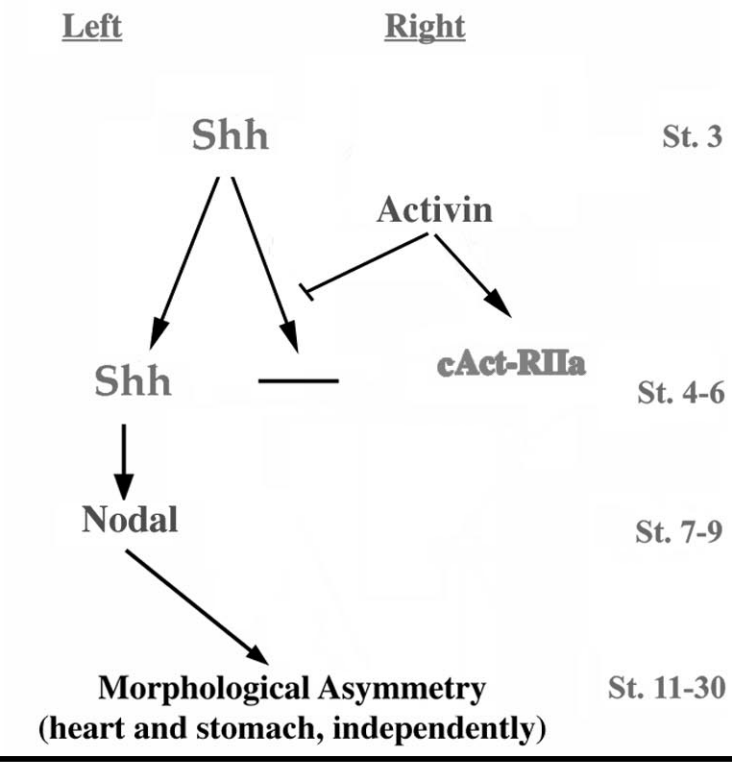


Figure 7: LR asymmetry and conjoined twins