LEFT-RIGHT ASYMMETRY IN ANIMAL DEVELOPMENT

William B. Wood
Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder CO 80309-0347; e-mail: wood@colorado.edu

KEY WORDS: chirality, embryonic axes, handedness, laterality, polarity, situs inversus

ABSTRACT
Most animal species exhibit left-right asymmetry in their body plans and show a strong bias for one handedness over the other. The mechanism of handedness choice, recognized as an intriguing problem over a century ago, is still a mystery. However, from recent advances in understanding when and how asymmetry arises in both invertebrates and vertebrates, developmental pathways for establishment and maintenance of left-right differences are beginning to take shape, and speculations can be made on the initial choice mechanism.

CONTENTS
THE PUZZLE OF HANDEDNESS BIAS ........................................ 54
Axes and Asymmetries in Animal Body Plans ............................ 54
Establishing and Maintaining Handed Asymmetry ..................... 55
Control of Embryonic Handedness ........................................ 56
L/R Asymmetry versus Bilateral Symmetry ............................... 56
CONTROL OF HANDEDNESS IN INVERTEBRATE EMBRYOS ........ 56
Handedness in Snails and Its Reversal .................................. 56
Handedness in Nematodes and Its Reversal ............................. 58
Drosophila: True Bilateral Symmetry? .................................. 62
CONTROL OF HANDEDNESS IN VERTEBRATE EMBRYOS .......... 64
Development of L/R Asymmetries in Vertebrates ...................... 64
Twinning and L/R Polarity Reversal ...................................... 71
CONCLUSIONS AND SPECULATIONS ..................................... 73
Mechanisms of Initial Handedness Choice ............................. 73
Bilateral Symmetry versus L/R Asymmetry ............................ 78
Only asymmetry can beget asymmetry.

F. R. Japp, 1898

...the contrast of left and right is connected with the deepest problems concerning the phylogenesis as well as the ontogenesis of organisms.

Hermann Weyl, 1952

THE PUZZLE OF HANDEDNESS BIAS

Axes and Asymmetries in Animal Body Plans

Animal body plans can be described in terms of three orthogonal axes: anterior-posterior (A/P), dorsal-ventral (D/V), and left-right (L/R). Although animals with clearly defined A/P and D/V axes are generally bilaterally symmetric, most also exhibit some internal L/R asymmetries. Familiar examples in humans include the placement of the heart, liver, and other viscera.

A consistent L/R asymmetry presents the possibility of two alternative mirror-image forms of the body plan, which are of opposite handedness but otherwise identical. L/R asymmetry itself is no more mysterious than A/P or D/V asymmetries, but how one handedness can be chosen over the other poses an intriguing problem. Most L/R-asymmetric species exhibit a bias, usually nearly absolute, for one enantiomeric form of the body plan over the other. Among humans, for example, the heart is (almost) always to the left of the midline and the liver to the right. How is the choice made?

To understand why this question is puzzling, consider sequential establishment of the three axes in a hypothetical embryo, starting with a spherically symmetric egg (Figure 1). The first axis to be determined, generally A/P, can pass through any point on the surface and its antipode; which of these becomes A and which becomes P does not affect the final geometry of the embryo (which now has A and P poles and an equatorial plane between). Orientation of the D/V axis is now constrained to be parallel to the equatorial plane, but it can still be established by choosing any point on the equator, and whether this point defines D or V also does not affect the final embryonic geometry. Orientation of the L/R axis is now further constrained to be parallel to only one diameter of the sphere, orthogonal to the other two axes. Which side will be called L and

1 First quotation from a lecture on Stereochemistry and Vitalism before the British Association, quoted by Weyl (1952, p. 31); second quotation from Weyl (1952, p. 38).

2 Some definitions: An asymmetric object and its mirror image, e.g. left and right hands, have the same pattern of asymmetry along their L/R axes but are of opposite handedness; they are called enantiomeric. A form that has a clear handedness, like a helix, screw, or spiral, is said to be chiral; such a form and its mirror image have opposite chirality (handedness).
which R is dictated by convention, but the polarity of the axis is not. Choice of this polarity determines the handedness of the embryo; it is the only axis for which orientation is completely predetermined, and selection of one polarity or the other affects the overall body plan. This choice is clearly not random. How and when is it made?

Establishing and Maintaining Handed Asymmetry

Many embryos exhibit handed L/R asymmetry, in morphology or differential gene expression or both, from early stages onward, indicating that polarity of the L/R axis is an early decision. There is direct evidence that this decision is made after A/P and D/V polarities have been established in nematodes (Priess & Thomson 1987), sea urchins (McCain & McClay 1994), and Xenopus (Danos & Yost 1995). Properties of conjoined twins, discussed further below, suggest that the same is true for birds and mammals.\(^3\)

In some invertebrate embryos, L/R polarity is clearly determined as early as second cleavage. In vertebrates, the first L/R asymmetry so far detected occurs later. Regardless of when L/R symmetry is broken, handedness must be dictated either (a) by external influences (in the parental uterus or the egg shell, for example) or (b) by the intrinsic handedness of some internal embryonic component. Once established, L/R polarity must be maintained throughout embryogenesis. The consequences of later ambiguities regarding L/R decisions, as occur in some pathologies of human visceral development, are severe dysfunction or death of the embryo.

\(^3\)In the discussion in the previous section, this order of axis determination was assumed; note, however, that the properties described for L/R apply to the third orthogonal axis established, whatever it may be.
Control of Embryonic Handedness

Embryonic handedness and its control were recognized as an intriguing puzzle more than a century ago (e.g., Crampton 1894, zur Strassen 1896). Early work on this problem is reviewed in an excellent monograph (Ludwig 1932), which was reprinted in 1970. During the past several years, partly stimulated by a wide-ranging Ciba Symposium on this topic (Bock & Marsh 1991), there has been a resurgence of interest in L/R asymmetry and considerable progress in documenting its cellular and molecular basis in several organisms, although the puzzle of handedness bias remains unsolved. This review discusses current understanding of L/R asymmetry in embryogenesis, with regard to some general questions: How does handed L/R polarity arise initially? How is it controlled genetically? Is it (unlike A/P and D/V polarity) dictated by a universal mechanism in diverse organisms? How and when is the initial cellular L/R asymmetry manifested in differential gene expression patterns on the two sides of the embryo? How are different patterns of gene expression and cell fates on the left and right maintained as complexity and cell number increase during development?

L/R Asymmetry versus Bilateral Symmetry

A related intriguing question, on which there is little evidence, regards the primacy of bilateral symmetry or L/R asymmetry; that is, which derives from the other? Is bilateral symmetry the ground state for embryos—expected to result from the ordered modulation of cleavage planes during cell proliferation in embryogenesis—so that L/R asymmetry must be imposed by special mechanisms? If so, why has such asymmetry evolved? Or is L/R-asymmetric embryogenesis the norm on which bilateral symmetry must be imposed during development? Extensive discussion of these questions is beyond the scope of this review, but they come up in several contexts below and are dealt with again briefly in conclusion (for more comprehensive treatment, see Jefferies 1991).

CONTROL OF HANDEDNESS IN INVERTEBRATE EMBRYOS

Handedness in Snails and Its Reversal

Gastropods such as the fresh-water snail Limnea have helical shells that usually coil with a right-handed (dextral) screw axis. Their early embryonic cell divisions show a pattern of spiral cleavage, in which the spindle axes of sister cells are tilted alternately clockwise and counterclockwise, so that successive cleavages give rise to tiers of cells that adopt a close packing with minimal surface area. The handedness of the tilt is normally dextral at second
Figure 2  Orientation of spindle axes and cleavage planes in early snail embryos. All embryos are oriented with the animal pole toward the viewer. Panels (a) and (c) are schematic representations of 2-cell embryos showing tilts of the spindles at mitosis: the broad end of each arrowhead represents the spindle pole that is tilted upward, i.e. toward the viewer. Panels (b, d, e) show 4-cell and 8-cell embryos of Limnea and Physa as drawn by Crampton (1894).

SINISTRAL MUTATIONS IN LIMNEA  Later breeding experiments between the common dextral and rare sinistral individuals of Limnea showed that sinistrality is inherited as a recessive maternal-effect mutation at a locus named sinistral (Sturtevant 1923), the wild-type function of which is required maternally for normal dextral spindle orientation at second cleavage and subsequent shell coiling. The alteration in this function resulting from sinistral mutant alleles causes not randomization of spindle orientation, but rather 100% reversal of handedness. Further genetic analysis of this phenomenon (Freeman & Lundelius 1982) showed that the so-called sinistral mutations arise and revert...
at a frequency higher than expected for point mutations and that the sinistral locus is probably complex. These authors presented genetic evidence for a model in which anomalous dextral and sinistral animals arise by mitotic or meiotic crossover events in the maternal germ line between two closely linked sites at this locus, both of which must be “+” on one chromosomal homologue during oogenesis for production of dextral offspring. They also reported the surprising finding that injection of ooplasm from dextral eggs into precleavage eggs from a sinistral parent caused the latter to cleave and develop dextrally! Unfortunately, neither the molecular genetics nor the biochemical nature of the dextralizing factor have been further investigated. However, these results suggest that the default mode may be sinistral cleavage, which is reversed by action of the normal sinistral gene product.

**Handedness in Nematodes and Its Reversal**

**ASYMMETRY IN ADULTS AND EMBRYOS**  
Nematodes are predominantly bilaterally symmetric, but they exhibit several internal L/R asymmetries (zur Strassen 1896, 1951, 1959). In the free-living species *Caenorhabditis elegans* these include positioning of the gonad and intestine in the pseudocoelom, placement of the coelomocytes and a few other cells, and the path and anatomy of the ventral nerve cord, in which cell bodies are on one side and processes on the other (Sulston & Horvitz 1977, Sulston et al 1983; reviewed in White 1988). These asymmetries have the normally invariant handedness shown in Figure 3a, which is arbitrarily referred to as dextral.

The origins of handed asymmetry, which first appears early in nematode embryogenesis (zur Strassen 1896), have been described in detail for *C. elegans*. The sperm entry point at fertilization defines the future posterior pole of the embryo and establishes its A/P polarity (Goldstein & Hird 1996). The 2-cell embryo appears to be radially symmetric around the A/P axis. D/V polarity is established at second cleavage as the elongating AB-cell spindle, originally orthogonal to the A/P axis, is forced by constraints of the egg shell to assume an oblique angle, with one spindle pole posterior to the other. The posterior spindle pole gives rise to the nucleus of the ABp cell, whose position in the resulting 4-cell embryo (Figure 3b; see legend for cell nomenclature) defines the future dorsal side of the embryo (Deppe et al 1978, Sulston et al 1983). D/V polarity can be experimentally reversed during this process by reorienting the AB spindle with a microneedle (Priess & Thomson 1987).

L/R asymmetry and polarity become apparent during the next cleavage, in which ABa and ABp divide in the L/R direction. The two spindles originally form parallel to the L/R axis, orthogonally to both the A/P and D/V axes, but then skew clockwise (as viewed from the dorsal side) in parallel just prior to
**Figure 3** Asymmetries in normal (dextral) *C. elegans* adult hermaphrodite and early embryos. (a) Adult hermaphrodite, ventral view: A, anterior; P, posterior; D, dorsal; V, ventral; L, (animal’s) left; R, (animal’s) right; XC, excretory cell; CCs, coelomocytes; G, gonad; VNC, ventral nerve cord; V, vulva. (b) Early embryos: stages and views as indicated. Sister cell pairs are indicated where convenient by a hash mark across the cleavage plane that separates them. Diagrams show four of the five somatic founder cells (AB, MS, E, C) and the germ line cells P2 and P3. Descendants of founder cells (e.g. AB) are named according to their relative a/p or l/r positions; e.g. ABa is the anterior daughter of AB, and ABal is the left daughter of ABa. For clarity in the diagrams of 6- and 8-cell embryos, the AB prefix has been omitted from the names of AB descendants (modified from Wood et al 1996).
cytokinesis, so that both spindle poles on the left are anterior to their respective opposite poles on the right. As a result, in the 6-cell embryo (Figure 3b), the two daughter cells on the left side, ABal and ABpl, lie anterior to their sister cells on the right, ABar and ABpr, respectively (Sulston et al 1983, Wood 1991). The subsequent EMS and P2 cell divisions, primarily A/P in direction, are constrained by the positions of the AB-derived cells to skew as well, so that the resulting 8-cell embryo is markedly L/R asymmetric (Figure 3b). This asymmetry persists throughout the cleavage stage but becomes less pronounced during later embryogenesis, as the embryo establishes bilateral symmetry (see below).

Does the early asymmetry of the cleavage stage dictate the handed asymmetries seen in adults? In his classic cell lineage studies on the large parasitic nematode *Ascaris megaloecephala*, zur Strassen (1896), using fixed preparations, observed that 2.5% of the embryos had reversed (sinistral) handedness, corresponding to a similar percentage of sinistral animals in adult populations. However, because embryos of this obligate parasite do not develop to adulthood in the laboratory, he was unable to demonstrate that reversed embryos give rise to reversed adults.

In *C. elegans*, such cause and effect was established with the demonstration that reversal of embryonic handedness at the 6-cell stage by micromanipulation (Wood 1991) results in apparently complete mirror-image development. Following a successful operation, all the normal L/R asymmetries examined were reversed during embryogenesis and larval growth to produce viable, healthy sinistral adults. Sinistral hermaphrodites could mate with dextral males to yield outcross progeny and were also self-fertile, producing normal numbers of progeny with normal dextral handedness. These results demonstrate that dextral handedness is genetically, rather than epigenetically determined, that it is not essential for viability, and that embryonic handedness at the 6-cell stage dictates the handedness of most if not all subsequent L/R asymmetries during development.

The mechanism by which the normal handedness bias might be established remains enigmatic. There is no apparent L/R asymmetry in the *C. elegans* 4-cell embryo. Moreover, reversal of the D/V axis by micromanipulation at the 3- to 4-cell stage does not result in L/R reversed embryos (Priess & Thomson 1987), indicating that L/R polarity is not established independently of D/V polarity prior to this time. Presumably, therefore, some internal component of the embryo with intrinsic chirality is responsible for the biased skewing of the ABa and ABp spindles during their cleavage to produce the observed invariant handedness. One such component could be the centrioles, but there is presently no evidence bearing on this possibility (Wood et al 1996; see Conclusions and Speculations).
Other nematodes differ from *C. elegans* in their degree of handedness invariance. Late in his career, zur Strassen (1951, 1959) examined embryos from eight diverse free-living and parasitic nematodes and found characteristic but very different frequencies of sinistral embryos, ranging from <0.1% in one species to 50% for another (the dung beetle parasite *Bradynema rigidum*). However, he found the same dextral embryonic handedness preference for all seven of the species that exhibited a bias.

A possible explanation for the frequency differences could be that constraints exerted by the egg shell can differ in the extent to which they reinforce the handedness bias. Among animals developing from early *C. elegans* embryos treated with chitinase to remove the egg shell, the frequency of reversed animals increases to about 5% (Wood & Kershaw 1991, Wood et al 1996). In these embryos, the configuration of the 4-cell stage appears quite similar to that seen normally in 4-cell *Ascaris* embryos (zur Strassen 1896). Other early workers claimed to have observed rotation of the AB quadrant of cells at the 6-cell stage in living *Ascaris* embryos, resulting in handedness reversal (e.g. Dunschen 1929). It may be that a less constraining egg shell in *Ascaris* allows occasional cell rearrangements in the early embryo that give rise to reversed handedness, and that the different reversal frequencies seen in other species could be at least partially explained by differences in egg shell constraints during early cleavage. A recent finding that low-temperature growth increases the reversal frequency in *C. elegans* can be interpreted as an effect on egg shell formation (Wood et al 1996).

**SINISTRAL NEMATODE SPECIES AND SINISTRAL MUTANTS** Although most nematodes appear to be primarily or exclusively dextral, there is at least one species, *Acrobeloides bodenheimeri*, in which non-interbreeding sinistral as well as dextral isolates or subspecies have been reported (Steiner 1936, Siddiqi et al 1992, Felix et al 1996). This situation is reminiscent of the snail genera *Limnea* and *Physa*, or dextral and sinistral subpopulations of *Limnea* (which can interbreed). It raises the question of whether similar influences on early cleavage patterns could be operating in both snails and worms (see Conclusions and Speculations).

Genetic analysis of handedness bias in *C. elegans* would obviously be highly desirable. Ongoing screens for mutants exhibiting either randomization or complete reversal of handedness without significant lethality have so far been unsuccessful, although mutation in at least one gene that affects spindle orientations primarily in the cleavages of ABa and ABp, where handedness is established, does lead to a much higher than normal percentage of reversed animals among survivors (D Bergmann, L Rose & WB Wood, unpublished results).
IMPOSITION OF BILATERAL SYMMETRY: MAINTAINING L/R DEVELOPMENTAL DIFFERENCES TO ELIMINATE L/R MORPHOLOGICAL DIFFERENCES

Except for the few L/R asymmetries in the adult body plan shown in Figure 3, nematodes are essentially bilaterally symmetric, with similar cells and structures at equivalent positions on both sides along the body axis. Given the striking asymmetry of the early embryo, bilateral symmetry must somehow be superimposed during subsequent embryogenesis. This means that the body plan cannot arise from identically programmed bilaterally symmetric cell lineages on the two sides of the embryo, with lineally homologous cells adopting equivalent fates (which is generally the rule in *C. elegans* development; Sulston 1983, Sulston et al 1983). Instead, there must be L/R differences in the specification of many of these homologues to compensate for the asymmetric arrangement of the early blastomeres and produce a bilaterally symmetric animal, as first pointed out by Sulston et al (1983). The extent of these differences can be seen by tracing the lineal origins of bilaterally equivalent cells on the two sides of the animal. As shown in Figure 4, the origins of many of these cells are quite different on the left and the right.

The embryonic reversal experiments cited above showed that these differences must be dictated by cell interactions after the 6-cell stage, rather than by segregation of cell-autonomous left and right determinants, which would have caused physically reversed embryos to develop abnormally (Wood 1991, Wood & Kershaw 1991). Subsequent cell ablation and genetic studies have indeed demonstrated that interactions between specific AB-derived and non-AB-derived blastomeres establish cell fate differences between lineally homologous cells on the two sides of the embryo, and the molecular bases for a few of these interactions have been identified (Hutter & Schnabel 1995). For example, at the 12-cell stage, because of the embryonic asymmetry, the MS founder cell contacts the hypodermal precursor cell ABalp on the left, but not its lineal homologue ABarp on the right. MS passes an inductive signal, mediated by the Notch homologue GLP-1 to ABalp, causing its fate to be different from that of ABarp. If the MS cell is ablated, or if the embryo is mutant for the *glp-1* gene, this signal is not passed, and both ABalp and ABarp adopt the ABarp fate (Hutter & Schnabel 1994, Gendreau et al 1994). Several later inductions have been shown to specify subsequent more minor L/R differences in the AB lineage (Bowerman et al 1992, Hutter & Schnabel 1995, Moskowitz & Rothman 1996).

**Drosophila: True Bilateral Symmetry?**

Another potentially valuable organism for genetic analysis of handedness determination would, of course, be *Drosophila*. The fruit fly, however, is almost entirely bilaterally symmetric throughout development. The only handed asymmetries reported so far are a twist in the gut and a rotation of the male sex organ,
Figure 4  Lineal relationships among 18 pairs of contralaterally analogous cells in the AB lineage of *C. elegans*. Pairs of cells shown opposite each other in the center of the diagram are analogous in both position and fate: the members of each pair occupy approximately equivalent positions on the left and right sides of the embryo at the time they are born (32-AB-cell to 128-AB-cell stages), and they give rise to nearly or completely identical numbers and types of progeny cells during subsequent development. Their ancestors, back to the 4-AB-cell stage, are represented in their approximate relative A/P positions, with cells on the left more anterior than their lineal homologues on the right. Note that analogues among ABp descendants in the seven most posterior pairs are also lineal homologues, whereas the remaining more anterior analogues all have different lineal ancestries on the two sides of the embryo (as can be seen from their cell names as well as from the lineage diagram). Four analogues are generated by divisions that cross the midline (based on Sulston et al 1983, Sulston 1983; reproduced with permission from Wood & Kershaw 1991).
so that phenotypic identification of reversed animals in mutant screens would be difficult. Based on failed attempts to select for L/R asymmetry in populations, it has been suggested that there is no L/R polarity in *Drosophila* embryos (Tuinstra et al. 1990).

**CONTROL OF HANDEDNESS IN VERTEBRATE EMBRYOS**

*Development of L/R Asymmetries in Vertebrates*

**MORPHOLOGICAL L/R ASYMMETRIES** Like nematodes, vertebrates are generally bilaterally symmetric with a few L/R asymmetries of nearly invariant handedness, most notably the placement of the visceral organs. Unlike nematodes, however, morphological asymmetry is not apparent during cleavage stages. In chick embryos, a transient slight asymmetry can be seen in Hensen’s node during gastrulation (stages 5-7; Hara 1978, Cooke 1995), but the first gross morphological L/R asymmetry in vertebrates arises only during organogenesis, when cardiac primordia migrating from either side of the embryo fuse at the midline to form an initially symmetrical heart tube, which then develops a dextral loop. Subsequently, mouse and chick embryos undergo an axial rotation toward the right, and further asymmetries become apparent in the placement of the gut and other viscera.

**GENETIC CONTROL OF HANDEDNESS** Several mutations have been described in mice and humans that affect the establishment of embryonic L/R polarity (laterality), causing either partial or complete reversal of the normal handed asymmetry of the viscera. Complete reversal (situs inversus viscerum) occurs at a frequency of at least 1 in 20,000 live human births, often, though not always, as a heritable condition. Complete situs inversus is often entirely benign and, therefore, not recognized until later in life (if at all), but partial reversal (heterotaxia) can lead to severe cardiac abnormalities and other visceral plumbing problems. The best-characterized human example of a heritable laterality defect is Kartagener’s syndrome, an autosomal recessive condition resulting in male sterility, bronchial problems, and randomization of laterality; that is, complete situs inversus in about 50% of afflicted individuals and normal situs (situs solitus) in the remainder (McKusick 1988). The basis for these phenotypes has been identified as lack of the outer dynein arms in cilia and flagella (Afzelius 1976). However, the ultrastructural defects differ among affected families, suggesting that the syndrome may represent a family of mutations affecting the outer dynein arms, which consist of many proteins. (For convenience, this syndrome is nevertheless referred to subsequently as if it were a single gene defect.) The defect causes lack of motility (dyskinesia) in sperm flagella and...
bronchial cilia and, in an unknown manner, randomization of embryonic handedness, but the mutant gene(s) have not been identified. Other human heritable laterality defects are even less well understood (e.g. Burn 1991, Brueckner et al 1991, Casey et al 1993).

In mice, the recessive spontaneous iv (inversus viscera) mutation on chromosome 12 also leads to randomization of handedness; about 50% of surviving homozygous animals have normal situs, and the rest exhibit situs inversus (Hummel & Chapman 1959, Layton 1976, Brueckner et al 1989, McGrath et al 1992). The randomization depends on embryonic and not maternal genotype, as shown by transfers of iv/iv embryos into +/+ mothers and vice versa (Brown et al 1990). In contrast, the inv (inversion of embryonic turning) mutation, resulting from a transgene insertion on chromosome 4, causes almost 100% of homozygous embryos to develop with reversed situs, although these embryos do not survive and may have other defects (Yokoyama et al 1993). inv is unique so far among mammalian mutations in causing 100% reversal of the L/R axis, reminiscent of the sinistral mutation in snails. Legless, also a mouse insertional mutation on chromosome 12, causes multiple asymmetric limb malformations and situs inversus with correlated variable expressivity (Schreiner et al 1993).

MOLECULAR EVIDENCE FOR EARLY L/R ASYMMETRY IN MOUSE AND CHICK EMBRYOS Recent molecular analyses have not only identified several more genes involved in the establishment of embryonic handedness, but have also demonstrated that L/R asymmetry, although not evident from morphology, is established in vertebrate embryos well before heart looping. The stage was set for this work by experiments showing that the handedness of heart looping and subsequent situs could be influenced by early surgical or pharmacological treatments. In the rat, adrenergic agonists applied during but not after gastrulation were shown to cause situs reversal (Fujinaga & Baden 1991a,b), and a similar effect on heart looping was demonstrated in chick embryos (Hoyle et al 1992). In Xenopus, perturbation of the blastocoel roof extracellular matrix (ECM) during early gastrulation was found to cause random orientation of the heart and other viscera (Yost 1992; see also Ludwig 1932, p. 381). These indications that handedness of the body plan is established during gastrulation led to a search for asymmetric expression of genes known to be involved in axial patterning at this stage. By in situ hybridization with molecular probes in chick embryos, Levin and coworkers (1995) demonstrated asymmetric expression, in a temporal sequence beginning at stage 4, of activin receptor Ila, Sonic hedgehog (Shh), and cNR-1, a homologue of mouse nodal. The winged-helix class transcription factor HNF-3β also showed a brief, transient asymmetry of expression during stage 4. Several other patterning genes tested were expressed symmetrically. Further, these authors showed that ectopic application of activin or expression
of Shh on the wrong side could alter heart situs. The results suggested a pathway (Figure 5b) in which activation of activin receptor IIa on the right side of the primitive streak leads to repression of Shh on the right, thereby restricting its expression to the left side of the streak, where it causes asymmetric induction of nodal. Subsequent work has shown that the activin Bβ gene is expressed asymmetrically on the right side at stage 3, strongly suggesting that it is the ligand that triggers this sequence of signals, which demonstrates the earliest L/R asymmetry so far in the chick embryo (Levin et al 1997). The products of all these genes are of known importance in axial patterning. Activin and Nodal, both members of the TGFβ superfamily of growth factors, and Shh, a vertebrate homologue of the Drosophila hedgehog gene product, are all secreted proteins likely to act non-cell-autonomously as signaling ligands.

---

**Figure 5** Proposed developmental pathways for establishment of normal laterality in mouse, chick, and Xenopus. Question marks indicate possible unidentified components based on the likelihood that these pathways are homologous. Parentheses enclose factors whose involvement or position is still tentative. See text for further explanation.
Another possible signaling molecule in this process could be retinoic acid (RA). Application of RA to the right precardiac field, but not to the left, caused randomization of heart looping, suggesting a normal role for RA signaling in specification of the left side (Smith et al. 1997). These investigators also observed statistically insignificant effects of RA application on the laterality of nodal (though not Shh) expression, consistent with the possibility that RA could act early in the pathway of Figure 5b.

Recent studies in the mouse have suggested the presence of a similar pathway and related it to the action of the iv and inv genes. A possible new component, the lefty gene on chromosome 1, encodes a divergent TGFβ superfamily member. It is first expressed symmetrically, but by 8 days, in embryos with a few pairs of somites, the protein is detected asymmetrically on only the left side of the primitive streak and in the left lateral plate mesoderm (Meno et al. 1996). In about half of iv homozygous embryos and about one fourth of the embryos produced by inv/+ x inv/+ matings (presumably the inv homozygotes), the Lefty protein is detected only on the right. A nodal::lacZ reporter allele has been used to show that, as in chick, nodal is expressed only on the left side of the node (Collignon et al. 1996). Moreover, in embryos from inv/+ x inv/+ matings, the sidedness of nodal expression corresponds to the direction of heart looping and embryonic turning: on the left in normal embryos and on the right in the approximately 20% of reversed, presumed inv homozygous embryos. In contrast to the chick studies, no asymmetry in either transcript or protein localization was seen for Shh or HNF-3β gene products. Lack of asymmetry for Shh expression has been reported for other vertebrates as well (Ekker et al. 1995, Collignon et al. 1996). Moreover, Shh−/− mice produced by targeted gene disruption exhibited no laterality defects (Chiang et al. 1996), despite major abnormalities in axial patterning as well as in establishment or maintenance of the notochord (see following section). In contrast, doubly heterozygous HNF-3β+ − nodal+−/− embryos exhibited LacZ staining on both left and right sides and frequent defects in visceral situs. Embryos with more staining on the left developed with normal situs; in embryos with apparently equal staining on both sides, situs was random. These results indicate a genetic interaction between HNF-3β and nodal, supporting a role for the former in establishment of L/R asymmetry. Similar results on the sidedness of nodal expression in mice and the nodal homologue Xnr-1 in Xenopus were obtained by in situ hybridization, which also was used to demonstrate that sidedness of mouse nodal expression is randomized in iv/iv and reversed in inv/inv embryos (Lowe et al. 1996). Together these studies show that the signaling events leading to asymmetric nodal expression are downstream of the iv and inv genes (Figure 5a), which could therefore be involved in the initial choice of L/R polarity.
EARLY L/R DETERMINANTS AND THE ROLE OF THE NOTOCHORD IN XENOPUS AND ZEBRAFISH  

The first evident morphological asymmetry in both *Xenopus* and zebrafish embryos is the dextral looping of the heart. Danos & Yost (1995) noted that when *Xenopus* anterior dorsal development is perturbed, either by UV irradiation during the first cell cycle or by injection of *Xwnt-8* DNA into dorsal blastomeres between the 4- and 32-cell stages, the resulting embryos not only fail to develop dorsal anterior structures (e.g., head, notochord) but also exhibit cardiac left-right reversals at a frequency that correlates with the extent of dorsal anterior defects, approaching 50% for severely defective embryos. The best correlation was observed with extent of anterior notochord development, which in turn reflects the amount of organizer activity (Stewart & Gerhart 1990). These results are therefore consistent with those from chick and mouse described above, which implicate gene expression in the organizer (node) region in establishment of L/R polarity.

Further evidence for notochord involvement has come from the finding that two zebrafish mutants, *no tail* (*ntl*) and *floating head* (*flh*), both of which lack notochord (Halpern et al 1993, 1995, Odenthal et al 1996, Stemple et al 1996), exhibit randomized laterality of cardiac looping (Danos & Yost 1996). Interestingly, both genes encode putative transcription factors: homologues of mouse *Brachyury* (Schulte-Merker et al 1994) and *Xenopus Xnot* (Halpern et al 1995), respectively. Moreover, experiments in *Xenopus* involving extirpation of notochord and explantation of cardiac primordia show that dextral cardiac looping requires the presence of dorsoanterior structures, including the notochord, during neural fold stages (Danos & Yost 1996).

The first molecular asymmetry in *Xenopus* embryos is found in the expression of the nodal homologue *Xnr-1* (Lowe et al 1996, Lohr et al 1997), which is seen to the left of the organizer region during gastrulation, as in mouse and chick embryos. However, an earlier L/R asymmetry had been noted in the mesoderm-inducing potential of the Nieuwkoop center (Boterenbrood & Nieuwkoop 1973), a group of dorsal blastomeres near the vegetal pole of the 32-cell embryo (Figure 6) that produces maternally encoded signals for mesoderm induction and formation of the organizer before embryonic transcription begins. To identify the molecular basis for this asymmetry, Hyatt and coworkers (1996) injected mRNAs (for proteins implicated in dorsoanterior axis formation) into these blastomeres on either the left or the right. They found that injection of mRNA for an activatable form of the TGFβ-related *Vg1* (*BVg1*) on the right caused randomization of cardiac and visceral L/R asymmetry as well as *Xnr-1* expression; injection on the left did not. Injection of several other mRNAs had no effect on L/R asymmetry, including *Vg1* and a mutant form of *BVg1* (*BVg1-2cx*), neither of which can be processed to the mature active molecule. However, an RNA encoding a dominant-negative truncated
Figure 6  Dorsal views of (a) 16-cell and (b) 32-cell Xenopus embryos, showing vegetal blastomeres (L and R) into which mRNAs were injected to assay for randomization of cardiac asymmetry. Large black dot represents Nile blue stain marking the cleavage plane that bisects the left and right sides at approximately the position of the future organizer region. See text for further explanation (modified from Hyatt et al 1996).

activin receptor (tAR), known to block Vg1 signaling in mesoderm induction assays (Kessler & Melton 1995), caused rates of cardiac inversion and Xnr-1 mislocalization that were considerably higher when injected on the left than on the right. The BVg1- and tAR-injected embryos exhibited normal dorsoanterior and notochord development, arguing against a secondary effect of Vg1 through effects on notochord formation. These results suggest that establishment of L/R asymmetry in Xenopus is mediated by asymmetric differential processing of maternally derived Vg1 precursor protein to the mature form, and that this event is upstream of asymmetric Xnr-1 expression. The findings, therefore, are similar to those obtained in chick and mouse, in that an activin-like signaling molecule appears responsible for controlling establishment of L/R polarity mediated by asymmetric expression of a nodal homologue (Figure 5c). An apparent difference is that in Xenopus, Xnr-1 expression is normally induced on the left by Vg1 activation on the same side, whereas in chick, nodal expression is likely to be restricted to the left by expression of activin Bβ on the right (Levin et al 1997).

In Xenopus, there is evidence that the activin signal may act through the notochord itself to control nodal expression and subsequent cardiac looping. Experiments cited above (Danos & Yost 1996) implicated the notochord in specifying the direction of heart looping. Subsequent timed extirpation and explantation experiments showed that dorsal midline structures, probably including
the notochord, repress *Xnr-1* expression in the right lateral plate mesoderm during closure of the neural tube, thereby restricting its expression to the left side prior to the specification of cardiac orientation (Lohr et al 1997). These authors also observed good correlation between the rates of symmetric or reversed *Xnr-1* expression patterns and cardiac reversal, both among normal embryos and those treated with UV during the first cell cycle to inhibit formation of dorsoanterior structures to varying degrees. Their results suggest that midline cells in *Xenopus* may be responsible for the asymmetric inhibitory role postulated for *Shh* expression in chick on *nodal* expression and that *Xnr-1* expression in turn controls cardiac looping (Figure 5c).

Because signaling molecules such as Shh and Nodal are able to move through considerable distances in developing tissues, their asymmetric restriction to one side or the other near the dorsal midline and their failure to cross the midline when ectopically expressed (Levin et al 1995) suggest the existence of a barrier, which seems likely to be required for generation and maintenance of laterality. Danos & Yost (1996) note that the notochord could be serving three functions in these processes: providing a source of asymmetric signals, acting as a midline barrier or sink for signaling molecules, and perhaps mediating alignment of ECM fibrils with which the cardiac primordia must interact for correct looping (Yost 1992). The above findings predict that mutations preventing normal notochord formation are likely to cause randomization of laterality as a secondary defect. Two examples are the zebrafish mutants *flh* and *ntl* described above; the mouse mutant *no turning* (Ewart et al 1996) could be another. It is curious that the *Shh* mutation in mouse does not appear to cause abnormal laterality (Chiang et al 1996).

**CONTROL OF LATERALITY IN ORGANOGENESIS** The asymmetric expression of *nodal* appears to dictate the handedness of heart looping and other subsequent asymmetries in organogenesis. The mechanisms are not yet clear, but components in this process are beginning to be identified. In *Xenopus*, as mentioned above, Yost (1992) showed that perturbation of the ECM lining the blastocoel roof during early gastrulation (by microsurgery, administration of RGD peptides, or treatment with heparinase) causes random orientation of the heart and other viscera. A similar effect was seen after application of proteoglycan synthesis inhibitors during early neurula stages when mesodermal cardiac progenitor cells are moving across the matrix to the ventral midline (Yost 1990). These results suggest that asymmetric *Xnr-1* expression could affect downstream signals communicated to organ primordial cells through ECM.

What are these downstream signals? In chick embryos, Isaac et al (1997) have shown that the *cSnR* gene, encoding a zinc-finger protein of the Snail family, is expressed asymmetrically in the right lateral mesoderm. Antisense
inhibition of this expression caused randomization of heart looping and sub-
sequent embryo rotation, but did not perturb the normal asymmetry of cNR-1 (nodal) expression. Ectopic expression of activin or Shh decreased the asymme-
try of both cSnR and nodal expression. Taken together, these results suggest that
cSnR acts downstream of or in parallel to nodal in determining laterality. Smith
et al (1997) have reported asymmetric expression of two ECM proteins—one
on the left, the other on the right, in the precardiac mesoderm—and have shown
that these asymmetries are abolished by prior application of RA to the right pre-
cardiac field. Also in chick, two novel related basic helix-loop-helix (bHLH)
proteins designated dHAND and eHAND have been identified as required for
cardiac looping (Srivastava et al 1995). The mouse dHAND and eHAND ho-
mologues are expressed primarily on the right and the left sides of the looping
heart, respectively. A dHAND null mutation results in complete absence of a
right ventricle (D Srivastava & E Olson, personal communication).
Control of laterality in the three so far best-characterized vertebrates is sum-
marized and compared in Figure 5. The pathways are fragmentary but similar
each to suggest that they will turn out to be homologous. All three clearly
involve expression during gastrulation of a nodal-like gene to the left of the mid-
line, which controls later laterality decisions. Other features that seem likely to
be common are involvement of activin signaling upstream and of ECM com-
ponents and the bHLH proteins eHAND and dHAND downstream of nodal.
Ordering of the early inv and iv functions in the mouse and their roles in the
initial handedness choice are discussed under Conclusions and Speculations.

Twinning and L/R Polarity Reversal

Laterality defects including complete situs inversus occur in some monozygotic
and conjoined human twins, as well as in spontaneously or induced conjoined
twin embryos in experimental animals. The findings described above provide
a plausible explanation for this phenomenon and its correlation with different
types of embryonic conjunction. For example, Spemann & Falkenberg (1919)
observed that in conjoined amphibian twins induced by medial constriction of
early newt embryos (Triton), laterality was normal in the twin on the left and
randomized in the twin on the right. This result is consistent with the prediction
from Xenopus that the asymmetrically (left-side) processed Vg1 required for
correct lateralization would be present in the left-half embryo and deficient in
the right-half following constriction (Hyatt et al 1996).

In spontaneously arising or induced twin chick embryos, the presence of
laterality defects depends on the orientation of the two primitive streaks (see
Figure 7). In head-to-head twins, where the two streaks oppose each other, no
laterality defects are seen, even if the twinning is induced by transverse cutting
of a normal blastodisc (Levin et al 1997). This result argues that laterality
Figure 7  Orientation of primitive streaks in development of conjoined twins and proposed molecular interactions leading to laterality defects in one twin of a conjoined pair. (a) Various orientations of a secondary streak relative to the primary streak (heavier line) give rise to twins conjoined as in (b) for humans or their chick counterparts. Dots at one end of each streak indicate the node. (b) Names and configurations of the four classes of human conjoined twins. (c) Postulated interactions of signaling molecules giving rise to laterality defects in the right twin, as often seen for dicephalic twins arising from parallel twin streaks. (d) Postulated interactions of signaling molecules giving rise to laterality defects in the left twin, as sometimes seen for thoracopagus twins arising from obliquely oriented twin streaks. See text for further description (modified from Levin et al 1996).

is streak-autonomous, determined only after establishment of A/P and D/V polarity. If left and right external cues were already present at the blastodisc stage, the secondary embryo with its A/P polarity reversed would be expected to also exhibit L/R reversal. When twins develop with parallel streaks, the embryo on the left develops normally, while the embryo on the right exhibits randomized laterality. This is consistent with the view that activin from the left embryo would likely inhibit the proposed Shh activation of $\text{nodal}$ on the left side of the adjacent right embryo, which would then express $\text{nodal}$ on neither side
(Figure 7c). Levin and coworkers (1996) showed that this prediction was borne out experimentally when Shh and nodal expression patterns were analyzed in parallel twin streaks by in situ hybridization. In obliquely oriented twins, where the two streaks are initially farther apart but grow toward each other, the opposite result was observed: randomization in the left twin and normal laterality in the right twin. In situ analysis showed that nodal was expressed on both sides of the left twin, but only on the left side (i.e. normally) in the right twin (Figure 7d). This result could be explained by induction of ectopic nodal expression in the left embryo by Shh protein produced in the left side of the right embryo as the streaks grow toward each other.

These results appear to explain the types of laterality defects observed in human conjoined twins, which are grouped into four classes on the basis of their orientation (Figure 7b). In the sample of 167 pairs of conjoined twins studied by Levin and coworkers (Levin et al 1996), none of those that would have originated from primitive streaks oriented end-to-end (craniopagi, joined only at the head; or ischiopagi, joined at the pelvis) exhibited laterality defects. In contrast, almost half of those that would have originated from parallel streaks (dicephali, joined laterally at the chest) or from obliquely oriented streaks (thoracophagi, joined obliquely at the chest or abdomen) exhibited reversal of heart situs in one of the two twins, usually the twin on the right. However, as also predicted by the model, laterality defects in the left twin were more frequent in thoracophagi (29%) than in dicephali (14%).

CONCLUSIONS AND SPECULATIONS

Mechanisms of Initial Handedness Choice

Intrinsic or Extrinsic? Is determination of handedness intrinsic to the embryo, or can it be imposed by asymmetries in the embryo’s external environment? In all cases where there is evidence bearing on this question, handedness determinants appear to be intrinsic and somehow dependent on the D/V axis. In nematodes (Priess & Thomson 1987) and sea urchins (McCain & McClay 1994), experimental reversal of the D/V axis also reverses the L/R axis. In Xenopus, establishment of laterality is dependent on generation of a complete dorsoanterior axial structure (Danos & Yost 1995), and in chick (Levin et al 1997) and human (Levin et al 1996), the absence of laterality defects in head-to-head twins argues against extrinsic determinants (Levin et al 1997), as discussed above.

Speculations on Intrinsic Mechanisms The enigma remains: how is L/R polarity initially established? Is it established by similar mechanisms in all embryos, or is it, like the A/P and D/V axes, established differently in different
From the conclusions above, we must begin with the assumption that laterality is initially dictated by the handed asymmetry of an internal component or process. A workable model for establishment of L/R differences in development must (a) identify this component or process, (b) explain how it can function to generate observed handed asymmetries, and (c) rationalize in terms of this component or process the known mutations that result in randomization or reversal of laterality.

Klar’s intriguing proposal to explain the inv mutation (Klar 1994), suggesting that asymmetry in the mouse embryo could be initially dictated by nonrandom segregation of nonequivalent sister chromatids “to specific daughter cells” early in development, fails to satisfy the first criterion: the direction of asymmetric segregation would have to be specified by an earlier decision dependent on a pre-existing asymmetry, which the model does not identify.4

Brown & Wolpert (1990) proposed that a chiral molecule or molecular complex could somehow be fixed in a particular orientation relative to the A/P and D/V axes (implying chirality of a midline structure) so that it could mediate asymmetric distribution of other components to provide initial specification of handedness. These authors also pointed out that choice of handedness is separable from generation of L/R asymmetry, which will still occur randomly in the absence of handedness choice, as appears to result from the Kartagener’s and iv mutations. This model, postulating handedness determination after many cells are present, is easier to visualize for vertebrate than for invertebrate embryos in which handedness already can be specified at the second (snails) or third (nematodes) cleavages. The model also has difficulty explaining the 100% asymmetry reversal resulting from the inv mutation, although there is precedent for a single mutation causing chirality reversal of a macromolecular structure in the case of bacterial flagella (Shimada et al 1975).

As a third model, I propose that centrioles or centrosomes, independently of their chirality, could specify the initial handedness choice in at least some organisms. The two centrosomes in each cell at mitosis are temporally distinguishable based on the histories of their constituent centriolar pairs (in most animal species). These are comprised of three generations of centrioles, which can be called grandmother (G), mother (M), and daughter (D). The centrosome in each cell following cytokinesis contains a centriolar pair, designated GM; during G, and S phases G and M separate and replicate to produce a GD and an MD centriolar pair. The two differ structurally (Rieder & Borisy 1981, Vorobjev

4Even if there is nonrandom segregation at each cleavage, as suggested below for centrioles, this process would place the appropriate chromosome in the appropriate cell at a particular location only in embryos with invariant cell lineages, like those of C. elegans, and unlike those of mammals. In C. elegans there is evidence that the chromosomes introduced by the sperm at fertilization segregate randomly (Ito & McGhee 1987).
as well as functionally, as demonstrated most clearly in algal basal bodies (Ruffer & Nultsch 1987, Beech et al 1988). During subsequent prophase, the GD and MD centrosomes migrate about 90° away from each other to opposite points on the nuclear membrane prior to the onset of metaphase. The plane of this migration, specification of which is not understood, dictates the orientation of the subsequent spindle that will be formed between the two centrosomes. This in turn determines the cleavage direction of the following cell division, except in cases where the spindle is specifically reoriented prior to division by interaction with the cell cortex (Hyman & White 1987, Hyman 1989).

Centrosomes seem especially attractive as possible handedness determinants in the snail embryo, in which the behavior of the two spindle poles in each cell during second cleavage is sufficient to determine the entire L/R asymmetry of the body plan. Following first cleavage to produce an anterior and a posterior blastomere, the spindles in each cell form perpendicularly to the preceding spindle orientation, but they become tilted before division, so that in the anterior cell of the more common dextrally cleaving embryo, the spindle pole on the embryo’s right is below the L/R axial plane, whereas in the posterior cell, the spindle pole on the left is below the plane (Figure 2a). In a sinistral Physa or Limnea sinistral mutant embryo (Figure 2c), the tilts are reversed (Crampton 1894). Nonrandom segregation of mature and immature centrioles to the two spindle poles could account for the handed rotations of the two spindles, which could be moved by attachment at either the mature or the immature end to the cell cortex in each cell. The 100% reversal resulting from sinistral mutations, which are recessive, suggests that sinistral cleavage is the ground state and that the dextral phenotype requires function of the wild-type sinistral gene product in order for the spindles to rotate appropriately.

To explain how such a mechanism might operate, let us consider a more explicit model. In the C. elegans 2-cell embryo, there is evidence that the spindle in the posterior P1 cell is pulled from a D/V to an A/P orientation by fibers that initially attach both spindle poles to a site on the anterior cortex (Hyman 1989). A tug-of-war appears to ensue, which one fiber wins, pulling the corresponding pole around toward the anterior. Suppose, in Limnea at the two-cell stage that (a) such a cortical attachment site forms in both cells on the vegetal midline; that (b) the GD centrosome has a stronger affinity than MD for this site; and that (c) GD centrioles have been segregated nonrandomly to the right spindle pole in the anterior (upper) cell and the left spindle pole in the posterior (pointed ends of spindles in Figure 2a).5 These spindle poles will be pulled ventrally in both

5 Horwich & Brueckner (1993) have also proposed involvement of cortical attachment sites in handedness determination. There is a precedent for nonrandom segregation of centrosome-like organelles in budding yeast, where the newer spindle pole body goes preferentially into the bud (Vallen et al 1992).
cells, giving rise to the common dextral orientation. The *sinistral* gene could encode a maternally supplied component, associating with GD centrosomes only, which confers higher affinity to the attachment site. Suppose, further, that if this component is lacking, as in a *sinistral* mutant, then the MD centrosomes have a higher affinity than the incomplete GD centrosomes, and sinistral orientation of the spindles will result. One can also imagine a different kind of mutation, for example, causing a defect in the cortical attachment site. The resulting predicted phenotype would be randomization of handedness (as for the *iv* or Kartagener’s mutations), with perhaps some inviable embryos. Thus these two genes would be required for separate processes: *sinistral* for what I will call L/R polarity assignment, and the product of the second hypothetical gene for what I will call choice execution. The first process specifies the intrinsic handedness that will be chosen if a choice is made; the second executes the choice. Together these two processes could constitute the mechanism of handedness choice that actually establishes a preferred or unique polarity for the L/R axis during development in a given species.

Similar informational mechanics could be operating to dictate handedness in nematodes. Laufer et al (1980) noted that an isolated *C. elegans* AB cell in culture divides with a spiral cleavage pattern, similar to that of *Limnea*, except that succeeding rounds of division spiral in the same direction to give a helical array of cells, rather than alternating as in the snail. Also, the handedness of this spiral and the tilt direction of the spindles beginning at the second AB cleavage are opposite to that of the corresponding features in dextral snails. Therefore, in a *C. elegans* embryo, after the anterior cell (ABA) divides, the daughter cell on the embryo’s left (ABAl) is located below the plane of the L/R axis, and the right daughter cell ABAr is located above. It seems likely that this cleavage pattern is the ground state for AB division and that in the intact embryo, AB cells follow this pattern to the extent that interactions with the egg shell and P1-derived blastomeres allow.6 In the 6-cell embryo, ABAl is in fact ventral to ABAr. The clockwise skewing of the ABa and ABp spindles during cleavage that causes ABAl also to be anterior to ABAr (Wood 1991) could perhaps result from downward force on the left spindle pole of ABa against the inclined plane provided by the underlying EMS cell (see Figure 3b), causing this pole to slide forward.

Could initial handedness choice be dictated by a similar mechanism in embryos with other cleavage patterns? In sea urchin and *Xenopus*, cleavage is equatorial and no cellular L/R asymmetry is apparent during the cleavage stage. In most species of both organisms, the first cleavage bisects the embryo at the dorsal midline, dividing it into left and right halves. At least in the sea urchin, however, L/R polarity is not yet established at this stage; embryos bisected into

---

6Note that the normal handedness of this pattern has been defined as dextral in *C. elegans*. 
right and left halves at this or several subsequent cleavage stages can still give rise to a pair of embryos with normal handedness (McCain & McClay 1994). The first handed structural asymmetry is seen only at the pluteus stage. Non-random centriolar segregation at first cleavage therefore seems unlikely as the mechanism for handedness determination.

In mammalian embryos, which undergo rotational cleavage, handedness also seems unlikely to be determined in early cleavage because chimeras made at later stages do not show the heterotaxia that might be expected if cells committed to left- or right-side fates were mixed. Moreover, among 21 aggregation chimeras made by combining 8-cell \textit{iv}/\textit{iv} and +/+ embryos, the majority developed with normal situs (Brown et al 1990). In mammals, however, we can also look to mutant phenotypes for clues to early events. The Kartagener and \textit{iv} mutations do not eliminate but simply randomize laterality; therefore, these genes function in choice execution. The 100% reversal seen in mouse embryos homozygous for the recessive \textit{inv} mutation suggests that this gene, like the \textit{sinistral} gene in snails, may normally function to reverse a ground-state L/R asymmetry that is opposite to the normal state in terms of absolute left and right. Therefore, like \textit{sinistral}, it functions in polarity assignment.

Can the \textit{inv}, \textit{iv}, and Kartagener’s gene(s) functions be ordered and placed in the pathway for establishment and maintenance of laterality in mammalian embryos (Figure 5a)? The functions of activin and \textit{nodal} are clearly downstream of the \textit{inv} and \textit{iv} genes in the mouse. Horwich & Brueckner (1993) point out that the degree of heterotaxia associated with laterality mutations should correspond to their position in such a pathway; a defect in the initial choice should result in only complete reversals or no reversal of the entire body plan, while defects in later steps would be more likely to affect some organs or parts of organs but not others. By this criterion, the Kartagener’s gene would be furthest upstream, followed by \textit{iv} and then \textit{inv}. However, if \textit{inv} is required for polarity assignment and \textit{iv} for choice execution as postulated, it would be predicted that in doubly homozygous mouse embryos, mutant for both genes, the \textit{iv} mutation would be epistatic; that is, the embryos would exhibit randomized laterality. This prediction is borne out by experiment (P Overbeek, personal communication; cited in Horwich & Brueckner 1993, Klar 1994). By the usual conventions of developmental genetics, this result indicates that \textit{iv} acts downstream of \textit{inv} as the model proposes.

Given that the defect in Kartagener’s syndrome may be in a motor protein, it is tempting to speculate that the normal function of the gene(s) could be involved in positioning of a chiral intracellular component, and we are back to the possibility of a role for the centriole (Horwich & Brueckner 1993). Perhaps, after all, it is also involved in vertebrate handedness choice through control of spindle orientations, although a mechanism is difficult to imagine at this point.
It is small comfort that there remains a cellular organelle about which so little is still known that it can serve as the basis for such speculation! We hope that this situation will change in the near future, allowing a general centriole-based model for handedness determination to be more rigorously evaluated. In addition, identification of the $iv$, $inv$, and Kartagener's gene products could provide important insight into how the initial handedness choice is made (see note added in proof).

**Bilateral Symmetry versus L/R Asymmetry**

It seems likely that external bilateral symmetry, in particular for aquatic organisms, could have adaptive value for efficient locomotion and, therefore, would have been selected for in evolution. In some embryos, such as those of the nematodes, early embryonic stages are highly asymmetric, and bilateral symmetry must be superimposed by later compensating adjustments of cell fates for lineal homologues on the two sides. However, in embryos with radial cleavage (e.g. sea urchins) or bilaterally symmetric cleavage (e.g. Ascidians), bilateral symmetry is present from the outset, and morphological L/R asymmetries appear to be added at later stages. As more molecular markers are discovered, it will be of interest to learn how early in these L/R-symmetric embryos asymmetry of gene expression can be detected. It will also be of interest to determine whether invertebrate homologues of vertebrate asymmetry markers can be found. If so, we can ask whether they are expressed with the same absolute handedness as in vertebrates, or whether the L/R axis, like the D/V axis (Holley et al. 1995, Hogan 1995), could have become inverted in the course of vertebrate evolution.

**ACKNOWLEDGMENTS**

I am grateful to S Dutcher, R McIntosh, and J Yost for helpful discussions, to members of my research group for suggestions and comments on the manuscript, and to M Brueckner, E Olson, P Overbeek, R Schnabel, C Tabin, and J Yost for communication of unpublished results. Research from the author's laboratory was supported by a National Institutes of Health grant (HD-29397).

---

**Literature Cited**


Boterenbrood EC, Nieuwkoop PD. 1973. The
Danos M, Yost J. 1995. Linkage of cardiac left-right asymmetry and dorsal-anterior development in Xenopus. Development 121:1467–74
Danos MC, Yost HJ. 1996. Role of notochord in specification of cardiac left-right orientation in Zebrabfish and Xenopus. Dev. Biol. 177:96–103
Hummel KP, Chapman DB. 1959. Visceral
Hutter H, Schnabel R. 1994. *glp-1* and induc-
tions establishing embryonic axes in *C. ele-
gans*. *Development* 120:2051–64

Hutter H, Schnabel R. 1995. Establishment of
left-right asymmetry in the *Caenorhabditis
elegans* embryo: a multistep process involve-
ning a series of inductive events. *Development*
121:3417–24

Hyatt B, Lohr J, Yost H. 1996. Initiation of ver-
tebrate left-right axis formation by maternal

Hyman A. 1989. Centrosome movement in the
early divisions of *Caenorhabditis elegans*:
a cortical site determining centrosome posi-

Hyman A, White J. 1987. Determination of
cell division axes in the early embryogene-
105:2123–35

Isaac A, Sargent MG, Cooke J. 1997. Control
of vertebrate left-right asymmetry by a snail-

Ito K, McGhee J. 1987. Parental DNA strands
segregate randomly during embryonic de-
velopment of *Caenorhabditis elegans*. *Cell*
49:329–36

Jeffries RPS. 1991. Two types of bilateral sym-
metry in the Metazoa: chordate and bilate-
rian. See Bock & Marsh 1991, pp. 94–127

Kessler DS, Melton DA. 1995. Induction of dor-
sal mesoderm by soluble, mature Vgl1 pro-
tein. *Development* 121:2155–64

the left-right axis in vertebrates. *Trends Genet.*
10:392–96

Laufer J, Bazzicalupo P, Wood W. 1980. Seg-
regation of developmental potential in early
embryos of *Caenorhabditis elegans*. *Cell*
19:569–77

Layton WM. 1976. Random determination of
a developmental process. *J. Hered.* 67:336–38

A molecular pathway determining left-right
asymmetry in chick embryogenesis. *Cell*
82:803–14

Levin M, Pagan S, Roberts DJ, Cooke J, Kuehn
MR, Tabin CJ. 1997. Different aspects of lat-
erality are independently controlled by an ap-
parently streak-autonomous signaling path-
way initiated by activin. *Development*. In
press

Levin M, Roberts DJ, Holmes LB, Tabin C.
1996. Laterality defects in conjoined twins.
*Nature* 384:324

Lohr JL, Danos MC, Yost HJ. 1997. Left-right
asymmetry of a nodal-related gene is regu-
lated by dorsoanterior midline structures dur-
ing *Xenopus* development. *Development*. In
press

Lowe LA, Supp DM, Sampath K, Yokoyama

T. Wright CVE, et al. 1996. Conserved left-
right asymmetry of nodal expression and al-
terations in murine *situs inversus*. *Nature*
381:158–61

Ludwig W. 1932. *Das Rechts-Links Problem
im Tierreich und beim Menschen*. Berlin:
Springer-Verlag. 496 pp. Reprinted 1970

McCaIN ER, McClay DR. 1994. The establish-
ment of bilateral asymmetry in sea urchin em-
bryos. *Development* 120:395–404

Duplication/deficiency mapping of *situs
inversus* viscerum (iv), a gene that determines
left-right asymmetry in the mouse. *Genomics*
14:643–48

McKusick VA, ed. 1988. *Mendelian Inherit-
ance of Man*, pp. 1023–25 (Entry 24468). Balti-
more: Johns Hopkins Univ. Press

Meno C, Saigoh Y, Fugii H, Ikeda M, Yokoyama
T, et al. 1996. Left-right asymmetric expres-
sion of the TGFβ-family member *lefty* in

Moskowitz IPG, Rothman JH. 1996. *lim-
12 and glp-1* are required zygotically for early
embryonic cellular interactions and are regu-
lated by maternal GLP-1 signaling in
*Caenorhabditis elegans*. *Development*
122:4105–17

Odenthal J, Haffter P, Vogelsang E, Brand M,
von Eeden FJM, Halpern SS. 1993. Correlation of
forelimb malforma-
tion asymmetries with visceral organ situs
in the transgenic mouse insertionional mutation

Schreiner CM, Scott WJ Jr, Supp DM, Potter
SS. 1993. Correlation of forelimb malforma-
tion asymmetries with visceral organ situs in
the transgenic mouse insertionional mutation

Schulte-Merker S, von Eeden FJM, Halpern
no tail (ntl) is the zebrafish homologue of the
mouse *Brachyury* gene. *Development*
120:1009–15

Left-handed to right-handed helix conver-
NOTE ADDED IN PROOF

Review of the literature for this article was concluded in March 1997. Since that time, the gene for an apparently cytoplasmic dynein heavy chain, with similarity to axonemal dyneins in the motor domain, has been positionally cloned as tightly linked to the mouse iv mutation and designated left/right dynein (lrd). The allelic iv and legless (lgl) mutations both cause lrd defects: In lgl mutants lrd is deleted, and in iv mutants a conserved glutamate in the development. Proc. R. Soc. London, Ser. B 241: 146–52


Yost H. 1990. Inhibition of proteoglycan synthesis eliminates left-right asymmetry in Xenopus laevis cardiac looping. Development 110:865–74


Lrd motor domain is replaced with lysine. The lrd mRNA is present in oocytes and early embryos (M Brueckner, personal communication; presented by DM Supp et al at the 62nd Cold Spring Harbor Symposium, *Pattern Formation During Development*, May, 1997). In addition, a YAC clone that rescues both the lethal and reversed laterality phenotypes resulting from the mouse inv mutation has been identified, giving promise that the gene(s) responsible will also soon be molecularly characterized (P Overbeek, personal communication).