SPECIAL ISSUE PERSPECTIVE

What's Left in Asymmetry?

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Left–right patterning is a fascinating problem of morphogenesis, linking evolutionary and cellular signaling mechanisms across many levels of organization. In the past 15 years, enormous progress has been made in elucidating the molecular details of this process in embryos of several model species. While many outside the field seem to believe that the fundamental aspects of this pathway are now solved, workers on asymmetry are faced with considerable uncertainties over the details of specific mechanisms, a lack of conceptual unity of mechanisms across phyla, and important questions that are not being pursued in any of the popular model systems. Here, we suggest that data from clinical syndromes, cryptic asymmetries, and bilateral gynandromorphs, while not figuring prominently in the mainstream work on LR asymmetry, point to crucial and fundamental gaps of knowledge about asymmetry. We identify 12 big questions that provide exciting opportunities for fundamental new advances in this field. ***Developmental Dynamics 237:3453–3463, 2008.*** **© 2008 Wiley-Liss, Inc.**

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"The great field for new discoveries . . . is always the unclassified residuum. Round about the accredited and orderly facts of every science there ever floats a sort of dust-cloud of exceptional observations . . . Any one will renovate his science who will steadily look after the irregular phenomena."
— William James, 1890

INTRODUCTION

Left–right (LR) asymmetry is an important and puzzling feature of embryogenesis (Burdine and Schier, 2000; Levin, 2005), also having important implications for human health and development (Cohen, 2001; Ramsdell, 2005). There now seems to be a perception outside the field that the question of LR patterning is largely solved. Cell biology textbooks and reviews now often summarize their view of the situation with one sentence, such as ". . . and ciliary movement also initiates Left-Right patterning". This is unfortunate, because it leads young developmental biologists (and reviewers of grant proposals) to think that there is little interesting science left in this field. However, those working on LR patterning know that there is considerable uncertainty about the early mechanisms of symmetry breaking, the evolutionary origins and degree of conservation of LR mechanisms at all levels, and the clinical implications of what we know and do not know about asymmetry (Tabin, 2005; Levin, 2006; Raya and Belmonte, 2006; Speder et al., 2007).

The study of LR patterning has largely been focused on the asymmetric gene cascades (Levin, 1998; Whitman and Mercola, 2001), understanding the dynamics of node cilia using technologically intricate approaches (Cartwright et al., 2004; Buceta et al., 2005), and modeling the properties of morphogen gradients of Nodal (Nakamura et al., 2006). However, a constellation of classic and recent observations suggests that, contrary to popular opinion, we are still missing major components of this field, and, in

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many ways, have been looking "where the light is good".

Here, we briefly sketch 12 major open questions in an effort to catalyze work on these challenging and novel directions. We chose these specific issues to be distinct in nature from the many details that remain to be fleshed out regarding the known pathways. The major areas of ignorance are revealed because they are not currently being addressed, have no established powerful model for their investigation, and/or involve a complex phenomenon not centered on a specific gene product. While we highlight some of the most difficult issues facing the field, these are also the areas of the greatest opportunity for discovery of new biology in this field.

(1) What is the molecular mechanism of "concordance" that determines whether a population is 50% *situs inversus* and 50% *situs solitus* vs. heterotaxic (random and independent sidedness of each organ)? Perturbations, like the spontaneous mouse mutants *iv* and *lgl* (Layton, 1976; McNeish et al., 1990; Singh et al., 1991), and patients with immotile cilia syndrome (Afzelius, 1976), exhibit 50% *situs inversus* and 50% *situs solitus*, while almost all of the known manipulations of early pathways (and mammalian mutations of Zic3, Lefty, cryptic/CFC, and ActRIIb) result in heterotaxia. None of the existing models (ciliary or intracellular) specifically address this; nor do they provide an explanation for how heterotaxia occurs. Thus, a more careful characterization of known mutations as to their degree of concordance (by scoring additional organ readouts and paying attention to subtle organ malformations as well as overall reversals), and the development of models that make predictions about the timing and nature of independent vs. linked *situs* decisions, will be essential.

(2) What is the base state of the LR axis? Complete inversions are induced by partial deletion of *inv* (Mochizuki et al., 1998), overexpression of mature Vg1 (Hyatt and Yost, 1998), and inhibition of serotonin receptor 3 (albeit with a lower penetrance; Fukumoto et al., 2005). The *inv* mouse is especially curious, because its mutation results in the majority of its offspring exhibiting *situs inversus* (although there is a detectable level of heterotaxia; Morgan et al., 1998; Otto et al., 2003). Unfortunately, the true genetic deletion has not been analyzed and all published experiments appear to have been carried out with the spontaneous mutant, in which a small part of the protein still exists (and hence may not be a true null). Thus, it remains to be determined whether this fragment is enough to bias asymmetry, refuting the bizarre possibility that the default state (without INV activity) is biased asymmetry, but in the reverse orientation, and that the INV gene product is needed to simply flip everything in the complete opposite direction. The *inv* mutation appears to affect a very early step, because a cross between an *inv* mouse and a Foxj1 knockout mouse (knockout Foxj1 mice are 50% *situs inversus* and 50% *situs solitus*; Chen et al., 1998; Brody et al., 2000; Zhang et al., 2004) produce mice that more closely resemble the Foxj1 phenotype (Tamakoshi et al., 2006). An understanding of the definitive *inv* loss-of-function should be very informative.

Recent studies show that retinoic acid signaling isolates somites from asymmetric cues (Vermot et al., 2005; Vermot and Pourquie, 2005). Symmetrical structures may inadvertently be susceptible to the effects of LR signaling molecules, because many developmentally important factors are used in patterning programs that overlap in space and time. The advantages of symmetry, however, have selected for the evolution of processes that shield the developing symmetric structures from asymmetric cues (Vermot et al., 2005; Vermot and Pourquie, 2005). Along with the orientation, it should be determined whether the base state of symmetrical structures is symmetry or asymmetry (Jefferies et al., 1996; Palmer, 2004).

(3) Are the breaking and orientation steps truly distinct and sequential? It is commonly thought that to pattern the LR axis, symmetry must first be broken and then oriented with respect to the other two axes (Brown and Wolpert, 1990). However, sometimes, perturbation of the same molecule in different locations can lead to predictable, differential laterality phenotypes. Ventral injection of chimeric *BVg1* into the R3 cells causes almost complete *situs inversus*, while injection into the R1 cell causes heterotaxia, and injections into the left side do not cause any significant laterality defects (Hyatt and Yost, 1998). These data may indicate a close relationship between the mechanisms for the breaking of symmetry and the biasing of asymmetry, and could serve as an entry point into this process. Precedents for such a bistable switch include the Notch/Delta system (Raya et al., 2004). Regardless of whether the breaking of symmetry and biasing of asymmetry is one step or two steps, the *iv* mutant mouse, which exhibits random 50% *situs inversus* and 50% *situs solitus*, indicates that the next processes of amplification and restriction of the initial asymmetric signal may still be able to receive cues from, or act downstream of the symmetry breaking point, and that the orientation mechanism may not be necessary to bridge the symmetry breaking point with concordant downstream organ morphogenesis. This makes sense evolutionarily; the direction of asymmetry is not as important as concordance between the organs, because *situs inversus* individuals exhibit few clinical symptoms, but heterotaxic individuals can often have severe medical problems (Burn, 1991; Peeters and Devriendt, 2006).

A related aspect is the precise characterization of phenotypes reported for the various genetic and pharmacological perturbations. To arrive at a complete model for LR patterning mechanisms, detailed characterization of the phenotypes caused by each experimental system is essential. Indeed, both *iv* and *inv* mouse phenotypes are not purely 50%/50% and fully inverted respectively, but include a significant degree of heterotaxias, isomerisms, and cardiac malformations (Hummel and Chapman, 1959; Morgan et al., 1998; Morishima et al., 1998; McQuinn et al., 2001). Likewise, *situs inversus* individuals are not really normal with respect to cardiac function because of discordances of orientation of structures at different scales (Delhaas et al., 2004; Ramsdell, 2005). Thus, the distinctions between symmetry breaking,

orientation, and concordance/morphogenesis may be more artifacts of our conceptual analysis of LR patterning phenotypes than a reflection of truly separate mechanisms during embryogenesis.

(4) Probably related is a set of puzzling inconsistencies about the universally conserved Left determinant *Nodal*, other asymmetric genes, and downstream readouts. If Nodal is indeed a Left determinant, why does bilateral Nodal not lead to left isomerism, but to randomization (Levin et al., 1997; Sampath et al., 1997)? What kind of mechanism could explain cases where the upstream markers exhibit a higher level of incorrectly sided expression then the downstream targets they induce in the LR cascade (Tsukui et al., 1999; Kitaguchi et al., 2000; Kelly et al., 2002; Levin et al., 2002; Morokuma et al., 2002), which may even become completely uncoupled from expression of *Nodal* (Cota et al., 2006)? Because the *iv* mouse exhibits 50% *situs inversus* and 50% *situs solitus*, we would expect nodal expression in the *iv* mouse population to be 50% left-sided, and 50% right-sided. Instead, *Nodal* in *iv* mice populations is expressed on the left-side, right-side, bilateral or absent, at statistically equal probabilities (Lowe et al., 1996). This finding indicates that, in *iv* mice, bilateral or absent *Nodal* does not prevent organ concordance. Yet, when left-sided *Nodal* is experimentally removed, heterotaxia does result (Brennan et al., 2002)! In zebrafish, over 30% of *cup* mutant embryos show complete *situs inversus*, but only a small percentage of them show right-sided *spaw* expression (Schottenfeld et al., 2007), even though *cup* is a homolog of *Pkd2* which is thought to be upstream of *Nodal* signaling. Another related puzzle: why do as many as 20% of siblings from a batch of embryos with 99% normal organ *situs* exhibit no expression of *Nodal* whatsoever (Levin and Mercola, 1998a; Takano et al., 2007)?

(5) How conserved are the early, intermediate, and late mechanisms in LR patterning? The various intracellular, biophysical, and ciliary mechanisms have each only been probed in a small number of representative model systems (Palmer, 2004; Levin, 2006; Levin and Palmer, 2007). While cilia appear to be involved in mouse (Nonaka et al., 1998; McGrath et al., 2003), zebrafish (Otto et al., 2003; Amack and Yost, 2004), and *Xenopus* (Schweickert et al., 2007), numerous other mechanisms such as cytoskeletal polarity (Thitamadee et al., 2002; Qiu et al., 2005), serotonergic signaling (Fukumoto et al., 2005), gap junctions (Levin and Mercola, 1998a, 1999; Chuang et al., 2007), and ion flows (Adams et al., 2006) are conserved in LR patterning among other phyla, including *Arabidopsis*, *Drosophila*, *Caenorhabditis elegans*, and ciliates (Levin and Palmer, 2007; Oviedo and Levin, 2007). The recent demonstration that individual cells can align the LR axis from intracellular polarity cues (Xu et al., 2007) suggests that subcellular and ancient chiral components of all cells may underlie an origin of LR patterning existing before the evolution of multicellularity. This powerful idea was presciently suggested in 1977, before the identification of any of the molecular aspects of asymmetry: "one may begin to look for a relationship between the bilateral symmetry of organisms and the mirror symmetry between daughter cells at an early state of the embryo" (Albrecht-Buehler, 1977).

The question of which, if any, of the early physiological mechanisms are used in mouse asymmetry is still open (see Levin and Palmer, 2007 for a discussion of alternative interpretations of the genetic data vis-a-vis the proposal of cilia as originators of asymmetry). Because of the ubiquitous compensation and redundancy that exists among ion transport machinery, a truly satisfactory approach to answering this would require knock-in of dominant negative and constitutively active transporter mutants that will change cell membrane potential in prestreak embryos in well-defined ways, rather than knockout of individual channel/pump genes. Related to this is the need to re-examine roles of "ciliary" proteins in mammals, as they are likely to have other functions in addition to ciliary roles, and hence, knockouts do not cleanly implicate a specific function of ciliary proteins in LR patterning. For example, *LRD* is expressed in a random manner in head folds (Supp et al., 1997), and, intriguingly, has been shown to be important for biased chromatid segregation (Armakolas and Klar, 2007).

(6) What explains the relatively low "penetrance" of some laterality-perturbing reagents and mutations? In a population where the background of defects is ~1%, a 25% incidence of heterotaxia in 1,000 embryos is unquestionably significant (Levin and Mercola, 1998a; Levin et al., 2002; Bunney et al., 2003). But, are the remaining embryos insensitive to the reagent? Are they somehow regulating to normalize asymmetry despite the effect of the reagent? Or are they truly part of an "affected" group of which 75% are developmentally determined to be *situs solitus*? With respect to pharmacological inhibition of ion channels and transporters, a possible reason for this may relate to the existence of robust physiological homeostasis mechanisms that can compensate for the loss of channel function, or even that the precise repertoire of functioning transporters expressed in a given individual may be a complex function of heredity, diet, and culture conditions that remain to be worked out. For example (unpublished observations in our lab), a pharmacological reagent from the same aliquot, on the same day, when applied to embryos of two different mothers, can cause heterotaxia rates of 3% to one group, and 55% in the other! This type of result reveals the complexity underlying the simple question of whether a particular pathway is or is not "involved in LR patterning."

One possible explanation for the variability in phenotypes may involve the early embryonic fate-map. Some organisms (e.g., *C. elegans*) have a very mosaic and stereotypical development; others (e.g., *Xenopus*) use a basic scheme in which blastomeres give rise to which tissues, but the exact cell boundaries vary considerably among individuals of normal egg batches. This is clearly seen in the original data sets of fate mapping studies (Dale and Slack, 1987; Moody and Kline, 1990; Chalmers and Slack,

2000). One prediction of this model is that a meta-analysis of LR perturbations among phyla should show more variability in phenotypic outcome in model species whose development is less stereotypical. If true, this would also suggest the existence of endogenous robustness mechanisms compensating for high levels of plasticity in embryonic cell fate (Palmer, 2004). Importantly, an understanding of these robustness mechanisms will also improve our ability to investigate LR patterning, because it is still not known why laboratory culture of rodent and flatfish embryos automatically results in a significant background of LR defects (Fujinaga et al., 1990; Fujinaga and Baden, 1991; Schreiber, 2006). The understanding of stability against, and susceptibility to, environmental factors in LR patterning will be a key component of a complete appreciation of the stochasticity underlying consistent asymmetry.

(7) What is the nature of "subtle" asymmetries? The field is almost entirely focused on pathways determining *situs* of heart and visceral organs, with some attention now being paid to laterality of the brain (Toga and Thompson, 2003; Halpern et al., 2005; Sun and Walsh, 2006; Hendricks and Jesuthasan, 2007). Central nervous system asymmetry may be patterned differently from that of body asymmetry, along pathways that may either be unrelated but parallel or, more likely, divergent from a common early step: although humans with *situs inversus* exhibit reversal of some anatomical brain asymmetries, they do not show reversals in functional asymmetries, and establish language dominance on the left cerebral hemisphere and a strong bias for right-handedness, just like in the general population (Kennedy et al., 1999; Tanaka et al., 1999; McManus et al., 2004). The zebrafish model system has not provided an ideal model for this discordance, because changes in canonical visceral *situs* pathways do seem to randomize fish brain asymmetry (Bisgrove et al., 2000; Concha et al., 2000; Barth et al., 2005; Essner et al., 2005), although point mutations of *cyclops* (the zebrafish *Nodal* homolog) result in normal heart looping and visceral *situs* but perhaps anatomical randomization in the brain because asymmetric gene expression is altered (Saude et al., 2005). However, the clinical data indicate that numerous other body structures and processes pay attention to LR cues, in pathways not predicted by or included in any of the current models (ciliary or intracellular).

There are several human syndromes that affect symmetrical or paired structures like the limbs (Smith et al., 1979), face or hips (Paulozzi and Lary, 1999), that consistently present more strongly on one side than the other. Facial clefting presents a consistent asymmetry (Delaney and Boyd, 2007), and this condition has recently become experimentally tractable, with the identification of molecular markers of asymmetry in symmetrical embryonic structures (somites; Golding et al., 2004a,b), and the discovery of a genetic entry point into zebrafish craniofacial asymmetry (Albertson and Yelick, 2005). Hemihyperplasia (Fraumeni et al., 1967; Clericuzio, 1993; Leung et al., 2002), a rare phenomenon where one side of the body to begins to grow in adulthood, is right-biased (Hoyme et al., 1998), and not only suggests that symmetric structures (knee joints, long bones of the legs) have LR identity, but that, astonishingly, they are able to retain that information for decades after embryogenesis.

Monozygotic twins are discordant for hemihypertrophy (West et al., 2003), and they display opposite-sided hair whorl direction, unilateral tooth defects, and other "bookending" phenomena (reviewed in Levin, 1999), strengthening the link between very early chiral decisions and subtle asymmetries in man. Is this related to the mirroring (Fig. 1) of cytoskeletal structures and migration paths observed when cells split (Albrecht-Buehler, 1977)? What is the molecular nature of the asymmetrical information that exists at early cleavages of vertebrate embryos and that results in asymmetry defects in one of the twins when they are separated early? Why does this separation result in opposite sidedness of subtle characteristics such as hair whorls in man (Golbin et al., 1993Aa,b; Morison et al., 1994) but also affect visceral and cardiac *situs* in some vertebrates (Mangold, 1921; Takano et al., 2007)? An entry point into the mechanisms of these subtle asymmetries may be provided by the study of hair whorls on the face of cattle produced by egg splitting (in the context of on-going in vitro production of valuable animals; Ozil et al., 1982; Gatica et al., 1984; Lanier et al., 2001; Meola et al., 2004). Does the fact that these subtle asymmetries in human monozygotic twins exist almost entirely above the neck

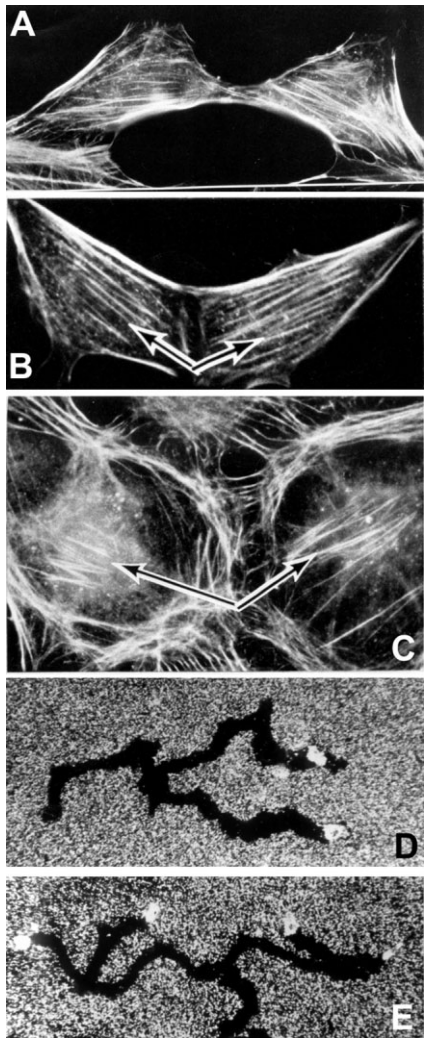

Fig. 1. Mirroring among single cells. **A–C:** Actin cytoskeletal elements of daughter cells in culture showing a mirroring of the angle of filament orientation. **D,E:** Sample migration tracks of cells in culture showing mirroring of cell paths after splitting. These data indicate the presence of chirality in single cells that is preserved at splitting, possibly providing a model for understanding enantiomer (bookending) of symmetry properties in monozygotic twins derived from early splitting of the egg. Images are used with permission of Albrecht Guenther-Buehler (Albrecht-Buehler, 1977).

suggest a separate LR-organizer for the head and brain, distinct from that for the body?

The link to sex determination pathways (which have no obvious asymmetry) is fascinating, revealed by the consistently sided presence of testes vs. ovaries in hermaphrodites (van Niekerk and Retief, 1981; Krob et al., 1994; Mittwoch, 2000, 2001, 2008). There is a cryptic asymmetry aspect to sex determination; for example, the consistent asymmetry in foot size is opposite between women and men (Levy and Levy, 1978). A model system may be available, because, in the Zic3 mutant, all affected males are heterotaxic, while females are wild-type or *situs inversus* (Gebbia et al., 1997). Finally, there are associations between asymmetry and the immune response (Renoux et al., 1983; Fride et al., 1990; Betancur et al., 1991; Neveu, 2002; Fu et al., 2003; Meador et al., 2004; Quaranta et al., 2004; Shen et al., 2005), and cancer (McManus, 1992; Sandson et al., 1992). The origin and implications of these links remain completely mysterious.

(8) "What is randomization"? This is a term often used in the field but attempting to construct a logical, mechanistic model of randomization quickly reveals our lack of conceptual clarity. For example, the normal chick embryo has a depolarization of cells on the left side of the streak followed by expression of *Sonic hedgehog* on the left side of the node. The simple way of modeling this would be to hypothesize that depolarization induces *Shh* expression. However, experimental induction of bilateral depolarization (Levin et al., 2002) does not result in bilateral *Shh*, but instead gives a spectrum of left-sided, right-sided, bilateral, and absent *Shh* expression. A mechanistic understanding of randomization would not only have to explain this bizarre result (which also occurs for other LR components), but would also have to show how cells are synchronized (so that the L and R domains of Hensen's node act as a unit, and do not exhibit a salt-and-pepper pattern on each side, reflecting cells' individual randomization). It would also need to predict the nonequiprobable outcomes (right-sided *Shh* is less frequent than bilateral *Shh* for example).

(9) What happens upstream of asymmetric gene expression in the chick? Before the right-sided expression of *Activin Receptor IIa* in the early chick streak (Levin et al., 1995; Stern et al., 1995), there are no nodal cilia, and the subsequent serotonin localization does not match the asymmetric pattern described in *Xenopus* (Fukumoto et al., 2005). Yet, many of the same molecular components are involved—serotonin and its receptors, gap junctions, and the H,K-ATPase/V-ATPase system (Levin, 2005). How are the same molecular signaling elements implemented in a body plan with a completely different large-scale architecture and cell size? Is the differential timing of similar physiological mechanisms among *Xenopus* and chick an instance of evolutionary heterochrony? Might the temporal sliding of molecular LR components relative to other embryonic events explain the evolutionary differences and similarities observed in asymmetry across phyla? Finally, what is the role of molecular chirality in amniotes, given that application of nonbiological stereoisomers of simple molecules induces heterotaxia in chick (Gray et al., 1942)?

(10) Is there a LR coordinator or organizer? In many types of embryos, LR patterning first takes place in a sheet of cells; it has been suggested that the information originates locally—in a kind of organizer or LR coordinator that is a single cell or small group of cells. This organizer is suggested to be in early blastomeres in *Xenopus* (Hyatt and Yost, 1998), in the node in mice (Pagan-Westphal and Tabin, 1998) and in the base of the primitive streak in chick (Levin and Mercola, 1998b). One proposal has been that LR patterning is a kind of planar cell polarity (Aw et al., 2008), as occurs in *Drosophila* wings and hair cells of the inner ear in mammals (Axelrod and McNeill, 2002; Zallen, 2007), and is a mechanism by which an organizer imposes a coherent LR directionality on the rest of the blastoderm. However, the ability of individual mammalian cells to set up a consistent LR polarity within a single cell (Xu et al., 2007), and the long-known ability of ciliates to epigenetically establish handedness (Nelsen et al., 1989) suggest that perhaps cells can independently orient their intracellular directionality along the LR axis. It will be crucial to determine whether LR information (including direction of the L and R sides, and position with respect to the midline) is imposed from without or generated internally in embryonic cells, or maybe even an interplay of both.

(11) Why can LR phenotypes be dissociated from essential housekeeping functions for numerous pathways? Several pathways that have been implicated in asymmetry also have important and profound roles in basic cell physiology. These include the V-ATPase H^+ pump (Adams et al., 2006), which acidifies vacuoles, gap junctions (Levin and Mercola, 1998a; Chuang et al., 2007), which regulate numerous aspects of small metabolites between cells, tight junctions (Brizuela et al., 2001; Eckert et al., 2005; Vanhoven et al., 2006), which provide epithelial integrity, K^+ channels (Levin et al., 2002), that regulate transmembrane potential, microtubules, and actin filaments (Thitamadee et al., 2002; Abe et al., 2004; Qiu et al., 2005; Adams et al., 2006; Aw et al., 2008), which are required for cell division and intracellular transport, and Ca^{++} signaling (McGrath et al., 2003; Raya et al., 2004), which regulates numerous second-messenger pathways. Despite the pleiotropic nature of these mechanisms, it has proven possible to analyze clean LR asymmetry phenotypes after loss-of-function perturbations of key members of these pathways. A priori, this is highly unexpected; blocking all K^+ channels with barium chloride or disrupting actin organization with Latrunculin would be expected to result in massive defects or toxicity, not the observed heterotaxia with normal dorsoanterior development. While this is not always possible, in the above cases a moderate level of abrogation could be identified and achieved, at which normal development and cell health was maintained, but the LR pathway was specifically disrupted. What signaling mechanisms or network properties in

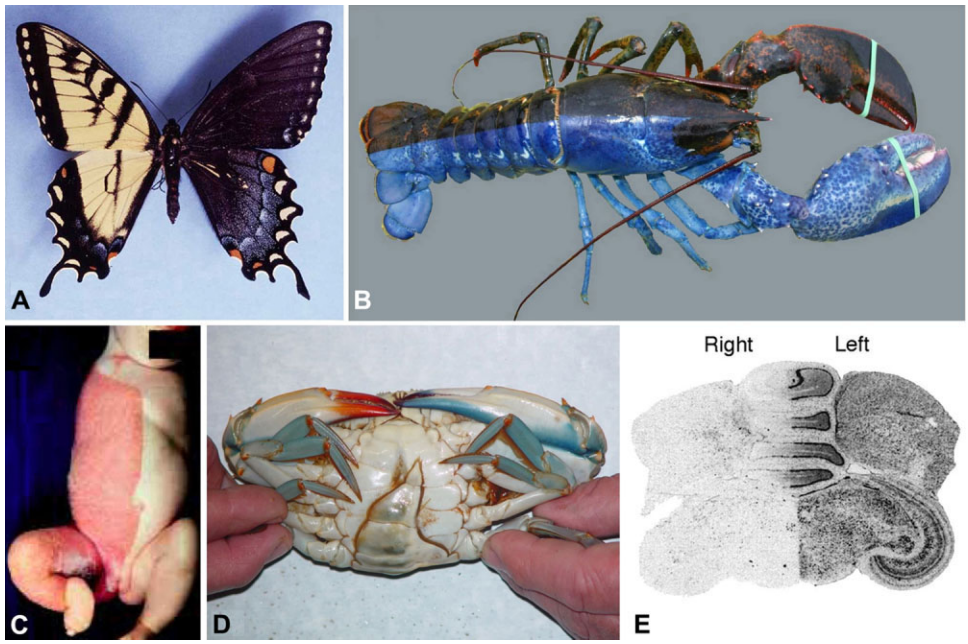

Fig. 2. Bilateral gynandromorphy. **A:** *Papilio glaucus*, used with permission from James K. Adams, showing boundary between male and female cells exactly down the midline. **B:** A bilateral gynandromorph lobster, used with permission of the Bangor Daily News; photo by Abigail Curtis. **C:** A CHILD syndrome patient with harlequin pigmentation, used with permission of John Wiley and Sons from the American Journal of Medical Genetics, 2000;90:340. **D:** A gynandromophic blue crab, *Callinectes sapidus* (ventral view; left side of the body exhibiting the narrower abdomen typical of males), with permission from Rom Lipcius (VIMS); taken from Kleps et al., (2007). **E:** A section of the brain of a gynandromorphic finch processed for in situ hybridization with probes to sex-specific transcripts (dark signal = female chromosome, no signal = male chromosome), where expression of a molecular marker in the brain of a gynandromorphic finch reveals that the separation between male and female cells is exactly down the midline. Image taken from Fig. 6 of (Agate et al., 2003), copyright held by National Academy of Sciences of the United States.

development allow LR patterning to be intimately dependent upon, and yet separable from, effectors of fundamental cell functions?

(12) When is the midline really determined? The left–right axis is defined with respect to the midline—a reflection line across which overall symmetry is preserved. There are many open questions about the mechanisms of midline determination, and this is clearly central to the question of LR patterning. In *Xenopus*, while the plane of first cleavage can be experimentally repositioned (Black and Vincent, 1988; Danilchik and Black, 1988), in normal embryos, the cleavage furrow usually corresponds to the future midline of the embryo (Klein, 1987; Masho, 1990). This makes it a natural candidate for LR mechanisms that rely on chirality (Danilchik et al., 2006) or redistribution of maternal intracellular components to the prospective L and R sides (Esser et al., 2006; Aw et al., 2008; Morokuma et al., 2008) during early cleavages. In contrast, the midline is thought to be established quite late in amniotes, when thousands of small cells exist and none have the advantage of spanning the midline of the whole embryo. If the midline (and thus position with respect to the LR axis) is not determined until cells are too small to enable transport to the whole embryo's L or R side, then initiation models based on multicellular signaling must be sought. Thus, knowing when the midline is truly set up is crucial for being able to gauge the plausibility of intracellular transport and planar cell polarity models in embryos of zebrafish, chick, and mouse.

The remarkable phenomenon of gynandromorphy throws doubt onto the certainty of late establishment of the midline in a wide range of phyla. In orthoptera, bilateral gynandromorphs are thought to result when one of the X chromosomes in an XX zygote is eliminated at the first cleavage division (Barranco et al., 1995), resulting in an animal that is part male and part female. The human cases revealed by X-linked genetic pigmentation syndromes (Happle et al., 1995) resemble examples of gynandromorphs found throughout phyla (Fig. 2), including butterflies, ants, crabs, crustaceans, and chickens (Farmer, 1972; Dang and Peterson, 1979; Homsher and Yunker, 1981; Sivaradjam and Bierne, 1981; Mey, 1982; Taber and Francke, 1986; Taylor, 1986; Micheli, 1991; Stevens and Munk, 1991; Barranco et al., 1995; Sagi et al., 1996, 2002; Moriyasu et al., 1998; Zou and Fingerman, 2000). What is truly remarkable is that the male–female division takes place precisely at the LR midline of the animal.

This is most strikingly seen in bird embryos, where the midline was always thought to be determined in a blastodisc of many thousands of cells. When failure of polar body extrusion occurs during meiosis (producing a binucleate ovum with Z and W nuclei), the resulting birds are chimeras of male and female cells (Lillie, 1931). Amazingly, the separation (revealed by the sex-specific pigmentation pattern) is exactly down the midline, and in roosters, is respected even by the comb on top of the head of the male half. One recent example is shown in Figure 2E, where expression analysis of a molecular marker in the brain of a

gynandromorphic finch reveals that the separation between male and female cells is exactly down the midline (Agate et al., 2003). A late definition of the midline would predict a random, mosaic distribution of male/female regions with respect to the axes of the adult. In contrast, the sharp midline separation suggests that the first cell cleavage may give rise to the L and R halves of the animal that can then inherit differential chiral information from the egg.

The main axes of rabbit embryos are known to be established before the appearance of the streak (Viebahn et al., 1995), and a similar situation has been proposed in the case of mouse development (Gardner, 2001; Piotrowska and Zernicka-Goetz, 2001; Plusa et al., 2002). Indeed, bilateral symmetry is said to be discernible in untreated living eggs and early embryos of the rat (Jones-Seaton, 1950; cited in Gardner, 1996). In human embryogenesis, the sharply unilateral pigmentation patterns that occur in humans with X-linked diseases such as CHILD syndrome requires unilateral X-inactivation (or the asymmetric activation or accumulation of some factor responsible for subsequent X-inactivation) to occur at two- or four-cell stage in such patients. This also suggests that the midplane is defined quite early in human embryos—a possibility consistent with the "bookending" phenomenon observed in monozygotic twinning (Levin, 1999). More research is clearly needed, to determine whether gynandromorphy is always bilateral, and to understand the mechanisms of midline determination in different amniote embryos. Mice also exhibit the strong sidedness of hermaphroditic organs observed in human cases, (Eicher and Washburn, 1983; Ward et al., 1987; Biddle et al., 1994), although the consistent laterality of testes' versus ovaries' placement is opposite of that observed in humans (van Niekerk and Retief, 1981; Krob et al., 1994).

If the chick embryo indeed sets up its midline at first cleavage, some of the puzzles regarding early mechanisms in the chick may need to be reformulated (part 9); the induction of *situs inversus* by division of the preincubation chick blastoderm (Lepori, 1967) is consistent with this model. However, even if set up very early, the midline can be experimentally respecified at later stages in mice and birds (Mintz, 1971; Eyal-Giladi and Fabian, 1980). A detailed study of the LR asymmetry phenotype of such animals has not been performed, and it remains to be determined exactly how heavily LR initiation relies on alignment between blastomere cleavage and the prospective midline in various model species. Indeed, even though *Xenopus* normally sets midline and asymmetry by the second cleavage, frog embryos *can* initiate correct LR patterning in organizers induced during blastula stages (Nascone and Mercola, 1997). Thus, while it is possible that some embryos establish a midline far earlier than previously thought, the models relying on intracellular transport across the prospective midline may need to be extended, to explain how LR patterning still occurs correctly when a new primary axis is initiated in fields of many small cells.

CONCLUSION

Left–right patterning is a truly fascinating aspect of developmental biology. Its implications extend to behavior (Klar, 1999; Crow, 2002; Bisazza and de Santi, 2003), immunology (Wise et al., 1993), and human culture (McManus, 2002). While tremendous progress has been made over the past 15 years, it is clear that major questions and fundamental areas of ignorance remain (especially with respect to subtle asymmetries and the timing of midline definition). There will be important and far-reaching discoveries to be made here for decades to come, and we hope that young scientists from physics, engineering, and cell biology enter the field, bringing fresh approaches to the contradictory data and brittle models existing today.

At the turn of the past century, Lord Kelvin identified "two small dark clouds on the horizon of classical physics"—at the time, minor observations that did not fit neatly into the powerful, unified paradigm of classical physics. Investigations of these anomalies gave rise to quantum theory and relativity. We hope that these open questions indeed contain several clouds of this type, leading to new discoveries, better questions, and perhaps ultimately a profound unification across scales of organization from quantum parity violations (Kondepudi, 1987; Mason, 1991) to large-scale patterning of a whole embryonic axis.

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REFERENCES

Abe T, Thitamadee S, Hashimoto T. 2004. Microtubule defects and cell morphogenesis in the lefty1lefty2 tubulin mutant of *Arabidopsis thaliana*. Plant Cell Physiol 45:211–220.

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

Afzelius BA. 1976. A human syndrome caused by immotile cilia. Science 193: 317–319.

Agate RJ, Grisham W, Wade J, Mann S, Wingfield J, Schanen C, Palotie A, Arnold AP. 2003. Neural, not gonadal, origin of brain sex differences in a gynandromorphic finch. Proc Natl Acad Sci U S A 100:4873–4878.

Albertson RC, Yelick PC. 2005. Roles for fgf8 signaling in left-right patterning of the visceral organs and craniofacial skeleton. Dev Biol 283:310–321.

Albrecht-Buehler G. 1977. Daughter 3T3 cells. Are they mirror images of each other? J Cell Biol 72:595–603.

Amack JD, Yost HJ. 2004. The T box transcription factor no tail in ciliated cells controls zebrafish left-right asymmetry. Curr Biol 14:685–690.

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

Aw S, Adams DS, Qiu D, Levin M. 2008. H,K-ATPase protein localization and Kir4.1 function reveal concordance of

three axes during early determination of left-right asymmetry. Mech Dev 125:353–372.

Axelrod JD, McNeill H. 2002. Coupling planar cell polarity signaling to morphogenesis. Sci World J 2:434–454.

Barranco P, Cabrero J, Camacho JPM, Pascual F. 1995. Chromosomal basis for a bilateral gynandromorph in *Pycnogaster Inermis* (Rambur, 1838) (Orthoptera, Tettigoniidae). Contrib Zool 65: 123–127.

Barth KA, Miklosi A, Watkins J, Bianco IH, Wilson SW, Andrew RJ. 2005. fsi zebrafish show concordant reversal of laterality of viscera, neuroanatomy, and a subset of behavioral responses. Curr Biol 15:844–850.

Betancur C, Neveu PJ, Vitiello S, Le Moal M. 1991. Natural killer cell activity is associated with brain asymmetry in male mice. Brain Behav Immun 5:162–169.

Biddle FG, Eisner JR, Eales BA. 1994. The testis-determining autosomal trait, Tda-1, of C57BL/6J is determined by more than a single autosomal gene when compared with DBA/2J mice. Genome 37:296–304.

Bisazza A, de Santi A. 2003. Lateralization of aggression in fish. Behav Brain Res 141:131–136.

Bisgrove BW, Essner JJ, Yost HJ. 2000. Multiple pathways in the midline regulate concordant brain, heart and gut left-right asymmetry. Development 127:3567–3579.

Black SD, Vincent JP. 1988. The first cleavage plane and the embryonic axis are determined by separate mechanisms in Xenopus laevis. II. Experimental dissociation by lateral compression of the egg. Dev Biol 128:65–71.

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

Brizuela BJ, Wessely O, De Robertis EM. 2001. Overexpression of the Xenopus tight-junction protein claudin causes randomization of the left-right body axis. Dev Biol 230:217–229.

Brody SL, Yan XH, Wuerffel MK, Song SK, Shapiro SD. 2000. Ciliogenesis and left-right axis defects in forkhead factor HFH-4-null mice. Am J Respir Cell Mol Biol 23:45–51.

Brown N, Wolpert L. 1990. The development of handedness in left/right asymmetry. Development 109:1–9.

Buceta J, Ibanes M, Rasskin-Gutman D, Okada Y, Hirokawa N, Izpisua-Belmonte JC. 2005. Nodal cilia dynamics and the specification of the left/right axis in early vertebrate embryo development. Biophys J 89:2199–2209.

Bunney TD, De Boer AH, Levin M. 2003. Fusicoccin signaling reveals 14-3-3 protein function as a novel step in left-right patterning during amphibian embryogenesis. Development 130:4847–4858.

Burdine R, Schier A. 2000. Conserved and divergent mechanisms in left-right axis formation. Genes Dev 14:763–776.

Burn J. 1991. Disturbance of morphological laterality in humans. CIBA Found Symp 162:282–296.

Cartwright JH, Piro O, Tuval I. 2004. Fluid-dynamical basis of the embryonic development of left-right asymmetry in vertebrates. Proc Natl Acad Sci U S A 101:7234–7239.

Chalmers AD, Slack JM. 2000. The Xenopus tadpole gut: fate maps and morphogenetic movements. Development Suppl 127:381–392.

Chen J, Knowles HJ, Hebert JL, Hackett BP. 1998. Mutation of the mouse hepatocyte nuclear factor/forkhead homologue 4 gene results in an absence of cilia and random left-right asymmetry. J Clin Invest 102:1077–1082.

Chuang CF, Vanhoven MK, Fetter RD, Verselis VK, Bargmann CI. 2007. An innexin-dependent cell network establishes left-right neuronal asymmetry in C. elegans. Cell 129:787–799.

Clericuzio CL. 1993. Clinical phenotypes and Wilms tumor. Med Pediatr Oncol 21: 182–187.

Cohen MM Jr. 2001. Asymmetry: molecular, biologic, embryopathic, and clinical perspectives. Am J Med Genet 101:292–314.

Concha ML, Burdine RD, Russell C, Schier AF, Wilson SW. 2000. A nodal signaling pathway regulates the laterality of neuroanatomical asymmetries in the zebrafish forebrain. Neuron 28:399–409.

Cota CD, Bagher P, Pelc P, Smith CO, Bodner CR, Gunn TM. 2006. Mice with mutations in Mahogunin ring finger-1 (Mgrn1) exhibit abnormal patterning of the left-right axis. Dev Dyn 235:3438–3447.

Crow TJ. 2002. Handedness, language lateralisation and anatomical asymmetry: relevance of protocadherin XY to hominid speciation and the aetiology of psychosis. Point of view [review]. Br J Psychiatry 181:295–297.

Dale L, Slack J. 1987. Fate map for the 32-cell stage of Xenopus laevis. Development 100:279–296.

Dang PT, Peterson BV. 1979. A case of bilateral gynandromorphism in Simulioum soubrense Vajime and Dunbar (Diptera: Simuliidae). Tropenmed Parasitol 30:548–550.

Danilchik MV, Black SD. 1988. The first cleavage plane and the embryonic axis are determined by separate mechanisms in Xenopus laevis. I. Independence in undisturbed embryos. Dev Biol 128:58–64.

Danilchik MV, Brown EE, Riegert K. 2006. Intrinsic chiral properties of the Xenopus egg cortex: an early indicator of left-right asymmetry? Development 133:4517–4526.

Delaney M, Boyd TK. 2007. Case report of unilateral clefting: is sonic hedgehog to blame? Pediatr Dev Pathol 10:117–120.

Delhaas T, Decaluwe W, Rubbens M, Kerckhoffs R, Arts T. 2004. Cardiac fiber orientation and the left-right asymmetry determining mechanism. Ann N Y Acad Sci 1015:190–201.

Eckert JJ, McCallum A, Mears A, Rumsby MG, Cameron IT, Fleming TP. 2005. Relative contribution of cell contact pattern, specific PKC isoforms and gap junctional communication in tight junction assembly in the mouse early embryo. Dev Biol 288:234–247.

Eicher EM, Washburn LL. 1983. Inherited sex reversal in mice: identification of a new primary sex-determining gene. J Exp Zool 228:297–304.

Essner JJ, Amack JD, Nyholm MK, Harris EB, Yost HJ. 2005. Kupffer's vesicle is a ciliated organ of asymmetry in the zebrafish embryo that initiates left-right development of the brain, heart and gut. Development 132:1247–1260.

Esser AT, Smith KC, Weaver JC, Levin M. 2006. Mathematical model of morphogen electrophoresis through gap junctions. Dev Dyn 235:2144–2159.

Eyal-Giladi H, Fabian BC. 1980. Axis determination in uterine chick blastodiscs under changing spatial positions during the sensitive period for polarity. Dev Biol 77:228–232.

Farmer A. 1972. A bilateral gynandromorph of Nephrops norvegicus. Mar Biol 15:344–349.

Fraumeni JF Jr, Geiser CF, Manning MD. 1967. Wilms' tumor and congenital hemihypertrophy: report of five new cases and review of literature. Pediatrics 40:886–899.

Fride E, Collins RL, Skolnick P, Arora PK. 1990. Immune function in lines of mice selected for high or low degrees of behavioral asymmetry. Brain Behav Immun 4:129–138.

Fu QL, Shen YQ, Gao MX, Dong J, Neveu PJ, Li KS. 2003. Brain interleukin asymmetries and paw preference in mice. Neuroscience 116:639–647.

Fujinaga M, Baden JM. 1991. Evidence for an adrenergic mechanism in the control of body asymmetry. Dev Biol 143:203–205.

Fujinaga M, Baden JM, Shepard TH, Mazze RI. 1990. Nitrous oxide alters body laterality in rats. Teratology 41:131–135.

Fukumoto T, Kema IP, Levin M. 2005. Serotonin signaling is a very early step in patterning of the left-right axis in chick and frog embryos. Curr Biol 15:794–803.

Gardner RL. 1996. Can developmentally significant spatial patterning of the egg be discounted in mammals? Hum Reprod Update 2:3–27.

Gardner RL. 2001. Specification of embryonic axes begins before cleavage in normal mouse development. Development Suppl 128:839–847.

Gatica R, Boland MP, Crosby TF, Gordon I. 1984. Micromanipulation of sheep morulae to produce monozygotic twins. Theriogenology 21:555–560.

Gebbia M, Ferrero GB, Pilia G, Bassi MT, Aylsworth A, Penman-Splitt M, Bird LM, Bamforth JS, Burn J, Schlessinger D, Nelson DL, Casey B. 1997. X-linked situs abnormalities result from mutations in ZIC3. Nat Genet 17:305–308.

Golbin A, Golbin Y, Keith L, Keith D. 1993a. Mirror imaging in twins: biological polarization—an evolving hypothesis. Acta Genet Med Gemellol (Roma) 42: 237–243.

Golbin A, Golbin Y, Keith L, Keith D. 1993b. Mirror imaging in twins: biological polarization. Acta Genet Med Gemellol 42:237–243.

Golding JP, Partridge TA, Beauchamp JR, King T, Brown NA, Gassmann M, Zammit PS. 2004a. Mouse myotomes pairs exhibit left-right asymmetric expression of MLC3F and alpha-skeletal actin. Dev Dyn 231:795–800.

Golding JP, Tsoni S, Dixon M, Yee KT, Partridge TA, Beauchamp JR, Gassmann M, Zammit PS. 2004b. Heparin-binding EGF-like growth factor shows transient left-right asymmetrical expression in mouse myotome pairs. Mech Dev Gene Expr Patterns 5:3–9.

Gray P, Cox GJ, Worthing H, Everhart WH, MacDonald E. 1942. The effect of stereo-isomeric substances on the heterotaxic rate in chick embryo. Anat Rec 82:416.

Halpern ME, Gunturkun O, Hopkins WD, Rogers LJ. 2005. Lateralization of the vertebrate brain: taking the side of model systems. J Neurosci 25:10351–10357.

Happle R, Mittag H, Kuster W. 1995. The CHILD nevus: a distinct skin disorder. Dermatology 191:210–216.

Hendricks M, Jesuthasan S. 2007. Asymmetric innervation of the habenula in zebrafish. J Comp Neurol 502:611–619.

Homsher PJ, Yunker CE. 1981. Bilateral gynandromorphism in Dermacentor andersoni (Acari: Ixodidae): morphologic and cytogenetic analysis. J Med Entomol 18:89–91.

Hoyme HE, Seaver LH, Jones KL, Procopio F, Crooks W, Feingold M. 1998. Isolated hemihyperplasia (hemihypertrophy): report of a prospective multicenter study of the incidence of neoplasia and review. Am J Med Genet 79:274–278.

Hummel KP, Chapman DB. 1959. Visceral inversion and associated anomalies in the mouse. J Hered 50:9–23.

Hyatt BA, Yost HJ. 1998. The left-right coordinator: the role of Vg1 in organizing left-right axis formation. Cell 93:37–46.

Jefferies RPS, Brown NA, Daley PEJ. 1996. The early phylogeny of chordates and echinoderms and the origin of chordate left-right asymmetry and bilateral symmetry. Acta Zool (Stockh) 77:101–122.

Jones-Seaton A. 1950. [Study of the cytoplasmic organization of the ovum of rodents, principally in regard to the ribonucleic basophilia.]. Arch Biol (Liege) 61: 291–444.

Kelly KA, Wei Y, Mikawa T. 2002. Cell death along the embryo midline regulates left-right sidedness. Dev Dyn 224: 238–244.

Kennedy D, O'Craven K, Ticho B, Goldstein A, Makris N, Henson J. 1999. Structural and functional brain asymmetries in human situs inversus totalis. Neurology 53:1260–1265.

Kitaguchi T, Nagai T, Nakata K, Aruga J, Mikoshiba K. 2000. Zic3 is involved in the left-right specification of the Xenopus embryo. Development Suppl 127:4787–4795.

Klar AJ. 1999. Genetic models for handedness, brain lateralization, schizophrenia, and manic-depression. Schizophr Res 39: 207–218.

Klein SL. 1987. The first cleavage furrow demarcates the dorsal-ventral axis in Xenopus embryos. Dev Biol 120:299–304.

Kleps RA, Myers TC, Lipcius RN, Henderson TO. 2007. A sex-specific metabolite identified in a marine invertebrate utilizing phosphorus-31 nuclear magnetic resonance. PLoS ONE 2:e780.

Kondepudi DK. 1987. Selection of molecular chirality by extremely weak chiral interactions under far-from-equilibrium conditions. Biosystems 20:75–83.

Krob G, Braun A, Kuhnle U. 1994. True hermaphroditism: geographical distribution, clinical findings, chromosomes and gonadal histology. Eur J Pediatr 153:2–10.

Lanier JL, Grandin T, Green R, Avery D, McGee K. 2001. A note on hair whorl position and cattle temperament in the auction ring. Appl Anim Behav Sci 73: 93–101.

Layton WM, Jr. 1976. Random determination of a developmental process: reversal of normal visceral asymmetry in the mouse. J Hered 67:336–338.

Lepori N. 1967. Orientation et situs viscerium et cordis chez les embryons jumeaux de cane. Monitore Zool Ital 1.

Leung AK, Fong JH, Leong AG. 2002. Hemihypertrophy. J R Soc Health 122: 24–27.

Levin M. 1998. Left-right asymmetry and the chick embryo. Semin Cell Dev Biol 9:67–76.

Levin M. 1999. Twinning and embryonic left-right asymmetry. Laterality 4:197–208.

Levin M. 2005. Left-right asymmetry in embryonic development: a comprehensive review. Mech Dev 122:3–25.

Levin M. 2006. Is the early left-right axis like a plant, a kidney, or a neuron? The integration of physiological signals in embryonic asymmetry. Birth Defects Res C Embryo Today 78:191–223.

Levin M, Mercola M. 1998a. Gap junctions are involved in the early generation of left-right asymmetry. Dev Biol 203:90–105.

Levin M, Mercola M. 1998b. The compulsion of chirality: toward an understanding of left- right asymmetry. Genes Dev 12:763–769.

Levin M, Mercola M. 1999. Gap junction-mediated transfer of left-right patterning signals in the early chick blastoderm is upstream of Shh asymmetry in the node. Development 126:4703–4714.

Levin M, Palmer AR. 2007. Left-right patterning from the inside out: widespread evidence for intracellular control. Bioessays 29:271–287.

Levin M, Johnson R, Stern C, Kuehn M, Tabin C. 1995. A molecular pathway determining left-right asymmetry in chick embryogenesis. Cell 82:803–814.

Levin M, Pagan S, Roberts DJ, Cooke J, Kuehn MR, Tabin CJ. 1997. Left/right patterning signals and the independent regulation of different aspects of Situs in the chick embryo. Dev Biol 189:57–67.

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

Levy J, Levy JM. 1978. Human lateralization from head to foot: sex-related factors. Science 200:1291–1292.

Lillie FR. 1931. Bilateral gynandromorphism and lateral hemihypertrophy in birds. Science 74:387–390.

Lowe LA, Supp DM, Sampath K, Yokoyama T, Wright CV, Potter SS, Overbeek P, Kuehn MR. 1996. Conserved left-right asymmetry of nodal expression and alterations in murine situs inversus. Nature 381:158–161.

Mangold O. 1921. Situs inversus bei Triton. Arch Entwickl-Mech Org 48:505–516.

Masho R. 1990. Close correlation between the 1st cleavage plane and the body axis in early Xenopus Embryos. Dev Growth Differ 32:57–64.

Mason SF. 1991. Origins of the handedness of biological molecules. Ciba Found Symp 162:3–10; discussion 10–15.

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

McManus IC. 1992. Reversed cerebral asymmetry and breast cancer. Lancet 339:1055.

McManus C. 2002. Right hand, left hand: the origins of asymmetry in brains, bodies, atoms and cultures. London: Weidenfeld and Nicolson.

McManus IC, Martin N, Stubbings GF, Chung EM, Mitchison HM. 2004. Handedness and situs inversus in primary ciliary dyskinesia. Proc R Soc Lond B Biol Sci 271:2579–2582.

McNeish JD, Thayer J, Walling K, Sulik KK, Potter SS, Scott WJ. 1990. Phenotypic characterization of the transgenic mouse insertional mutation, legless. J Exp Zool 253:151–162.

McQuinn TC, Miga DE, Mjaatvedt CH, Phelps AL, Wessels A. 2001. Cardiopulmonary malformations in the inv/inv mouse. Anat Rec 263:62–71.

Meador KJ, Loring DW, Ray PG, Helman SW, Vazquez BR, Neveu PJ. 2004. Role of cerebral lateralization in control of immune processes in humans. Ann Neurol 55:840–844.

Meola MG, Grandin T, Burns P, Deesing M. 2004. Hair whorl patterns on the bovine forehead may be related to breeding soundness measures. Theriogenology 62: 450–457.

Mey W. 1982. A bilateral gynandromorph of Anabolia-Furcata Brauer (Insecta, Trichoptera). Zool Anz 209:394–396.

Micheli F. 1991. Bilateral gynandromorph of the fresh-water crab potamon- fluvia-

tile herbst (Decapoda, Brachyura). J Crust Biol 11:561–568.
Mintz B. 1971. Genetic mosaicism in vivo: development and disease in allophenic mice. Fed Proc 30:935–943.
Mittwoch U. 2000. Genetics of sex determination: exceptions that prove the rule. Mol Genet Metab 71:405–410.
Mittwoch U. 2001. Genetics of mammalian sex determination: some unloved exceptions. J Exp Zool 290:484–489.
Mittwoch U. 2008. Different gene expressions on the left and the right: a genotype/phenotype mismatch in need of attention. Ann Hum Genet 72:2–9.
Mochizuki T, Saijoh Y, Tsuchiya K, Shirayoshi Y, Takai S, Taya C, Yonekawa H, Yamada K, Nihei H, Nakatsuji N, Overbeek PA, Hamada H, Yokoyama T. 1998. Cloning of inv, a gene that controls left/right asymmetry and kidney development. Nature 395:177–181.
Moody SA, Kline MJ. 1990. Segregation of fate during cleavage of frog (Xenopus laevis) blastomeres. Anat Embryol 182: 347–362.
Morgan D, Turnpenny L, Goodship J, Dai W, Majumder K, Matthews L, Gardner A, Schuster G, Vien L, Harrison W, Elder FF, Penman-Splitt M, Overbeek P, Strachan T. 1998. Inversin, a novel gene in the vertebrate left-right axis pathway, is partially deleted in the inv mouse. Nat Genet 20:149–156.
Morishima M, Yasui H, Nakazawa M, Ando M, Ishibashi M, Takao A. 1998. Situs variation and cardiovascular anomalies in the transgenic mouse insertional mutation, inv. Teratology 57:302–309.
Morison D, Reyes C, Skorodin M. 1994. Mirror-image tumors in mirror-image twins. Chest 106:608–610.
Moriyasu M, Mallet P, Comeau M, Benhalima K, Ghica O. 1998. Occurrence of pseudohermaphroditism in the rock crab, Cancer irroratus Say, 1817, in the southern Gulf of St. Lawrence, Canada (Decapoda, Brachyura). Crustaceana 71: 655–662.
Morokuma J, Ueno M, Kawanishi H, Saiga H, Nishida H. 2002. HrNodal, the ascidian nodal-related gene, is expressed in the left side of the epidermis, and lies upstream of HrPitx. Dev Genes Evol 212: 439–446.
Morokuma J, Blackiston D, Levin M. 2008. KCNQ1 and KCNE1 K+ channel components are involved in early left-right patterning in Xenopus laevis embryos. Cell Biochem Biophys (in press).
Nakamura T, Mine N, Nakaguchi E, Mochizuki A, Yamamoto M, Yashiro K, Meno C, Hamada H. 2006. Generation of robust left-right asymmetry in the mouse embryo requires a self-enhancement and lateral-inhibition system. Dev Cell 11:495–504.
Nascone N, Mercola M. 1997. Organizer induction determines left-right asymmetry in Xenopus. Dev Biol 189:68–78.
Nelsen EM, Frankel J, Jenkins LM. 1989. Non-genic inheritance of cellular handedness. Development 105:447–456.
Neveu PJ. 2002. Cerebral lateralization and the immune system. Int Rev Neurobiol 52:303–323.
Nonaka S, Tanaka Y, Okada Y, Takeda S, Harada A, Kanai Y, Kido M, Hirokawa N. 1998. Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. Cell 95:829–837.
Otto EA, Schermer B, Obara T, O'Toole JF, Hiller KS, Mueller AM, Ruf RG, Hoefele J, Beekmann F, Landau D, Foreman JW, Goodship JA, Strachan T, Kispert A, Wolf MT, Gagnadoux MF, Nivet H, Antignac C, Walz G, Drummond IA, Benzing T, Hildebrandt F. 2003. Mutations in INVS encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. Nat Genet 34:413–420.
Oviedo NJ, Levin M. 2007. Gap junctions provide new links in left-right patterning. Cell 129:645–647.
Ozil JP, Heyman Y, Renard JP. 1982. Production of monozygotic twins by micromanipulation and cervical transfer in the cow. Vet Rec 110:126–127.
Pagan-Westphal S, Tabin C. 1998. The transfer of left-right positional information during chick embryogenesis. Cell 93: 25–35.
Palmer AR. 2004. Symmetry breaking and the evolution of development. Science 306:828–833.
Paulozzi LJ, Lary JM. 1999. Laterality patterns in infants with external birth defects. Teratology 60:265–271.
Peeters H, Devriendt K. 2006. Human laterality disorders. Eur J Med Genet 49: 349–362.
Piotrowska K, Zernicka-Goetz M. 2001. Role for sperm in spatial patterning of the early mouse embryo. Nature 409: 517–521.
Plusa B, Piotrowska K, Zernicka-Goetz M. 2002. Sperm entry position provides a surface marker for the first cleavage plane of the mouse zygote. Genesis 32: 193–198.
Qiu D, Cheng SM, Wozniak L, McSweeney M, Perrone E, Levin M. 2005. Localization and loss-of-function implicates ciliary proteins in early, cytoplasmic roles in left-right asymmetry. Dev Dyn 234: 176–189.
Quaranta A, Siniscalchi M, Frate A, Vallortigara G. 2004. Paw preference in dogs: relations between lateralised behaviour and immunity. Behav Brain Res 153:521–525.
Ramsdell AF. 2005. Left-right asymmetry and congenital cardiac defects: getting to the heart of the matter in vertebrate left-right axis determination. Dev Biol 288: 1–20.
Raya A, Belmonte JC. 2006. Left-right asymmetry in the vertebrate embryo: from early information to higher-level integration. Nat Rev Genet 7:283–293.
Raya A, Kawakami Y, Rodriguez-Esteban C, Ibanes M, Rasskin-Gutman D, Rodriguez-Leon J, Buscher D, Feijo JA, Izpisua Belmonte JC. 2004. Notch activity acts as a sensor for extracellular calcium during vertebrate left-right determination. Nature 427:121–128.
Renoux G, Biziere K, Renoux M, Guillaumin JM, Degenne D. 1983. A balanced brain asymmetry modulates T cell-mediated events. J Neuroimmunol 5:227–238.
Sagi A, Khalaila I, Barki A, Hulata G, Karplus I. 1996. Intersex red claw crayfish, Cherax quadricarinatus (von Martens): functional males with pre-vitellogenic ovaries. Biol Bull 190:16–23.
Sagi A, Manor R, Segall C, Davis C, Khalaila I. 2002. On intersexuality in the crayfish Cherax quadricarinatus: an inducible sexual plasticity model. Invertebr Reprod Dev 41:27–33.
Sampath K, Cheng AM, Frisch A, Wright CV. 1997. Functional differences among Xenopus nodal-related genes in left-right axis determination. Development Suppl 124:3293–3302.
Sandson TA, Wen PY, LeMay M. 1992. Reversed cerebral asymmetry in women with breast cancer. Lancet 339:523–524.
Saude L, Lourenco R, Goncalves A, Palmeirim I. 2005. terra is a left-right asymmetry gene required for left-right synchronization of the segmentation clock. Nat Cell Biol 7:918–920.
Schottenfeld J, Sullivan-Brown J, Burdine RD. 2007. Zebrafish curly up encodes a Pkd2 ortholog that restricts left-side-specific expression of southpaw. Development 134:1605–1615.
Schreiber AM. 2006. Asymmetric craniofacial remodeling and lateralized behavior in larval flatfish. J Exp Biol 209:610–621.
Schweickert A, Weber T, Beyer T, Vick P, Bogusch S, Feistel K, Blum M. 2007. Cilia-driven leftward flow determines laterality in Xenopus. Curr Biol 17:60–66.
Shen YQ, Hebert G, Moze E, Li KS, Neveu PJ. 2005. Asymmetrical distribution of brain interleukin-6 depends on lateralization in mice. Neuroimmunomodulation 12:189–194.
Singh G, Supp DM, Schreiner C, McNeish J, Merker HJ, Copeland NG, Jenkins NA, Potter SS, Scott W. 1991. legless insertional mutation: morphological, molecular, and genetic characterization. Genes Dev 5:2245–2255.
Sivaradjam S, Bierne J. 1981. Sex differentiation in bilaterally allophenic animals produced by cloning of two bipartite male/female chimaeras of Lineus sanguineus. J Embryol Exp Morphol 65:173–184.
Smith AT, Sack GH, Jr., Taylor GJ. 1979. Holt-Oram syndrome. J Pediatr 95:538–543.
Speder P, Petzoldt A, Suzanne M, Noselli S. 2007. Strategies to establish left/right asymmetry in vertebrates and invertebrates. Curr Opin Genet Dev 17:351–38.
Stern C, Yu R, Kakizuka A, Kintner C, Mathews L, Vale W, Evans R, Umesono K. 1995. Activin and its receptors during gastrulation and the later phases of me-

soderm development in the chick embryo. Dev Biol 172:192–205.
Stevens BG, Munk JE. 1991. Lateral asymmetry in the thoracic segmentation of a king crab, Paralithodes-Camtschaticus (Tilesius, 1815) (Decapoda, Anomura), from Kodiak, Alaska. Crustaceana 61:317–320.
Sun T, Walsh CA. 2006. Molecular approaches to brain asymmetry and handedness. Nat Rev Neurosci 7:655–662.
Supp DM, Witte DP, Potter SS, Brueckner M. 1997. Mutation of an axonemal dynein affects left-right asymmetry in inversus viscerum mice. Nature 389:963–966.
Taber SW, Francke OF. 1986. A bilateral gynandromorph of the Western harvester ant, Pogonomyrmex-Occidentalis (Hymenoptera, Formicidae). Southwest Natural 31:274–276.
Tabin C. 2005. Do we know anything about how left-right asymmetry is first established in the vertebrate embryo? J Mol Histol 36:317–323.
Takano K, Ito Y, Obata S, Oinuma T, Komazaki S, Nakamura H, Asashima M. 2007. Heart formation and left-right asymmetry in separated right and left embryos of a newt. Int J Dev Biol 51:265–272.
Tamakoshi T, Itakura T, Chandra A, Uezato T, Yang Z, Xue XD, Wang B, Hackett BP, Yokoyama T, Miura N. 2006. Roles of the Foxj1 and Inv genes in the left-right determination of internal organs in mice. Biochem Biophys Res Commun 339:932–938.
Tanaka S, Kanzaki R, Yoshibayashi M, Kamiya T, Sugishita M. 1999. Dichotic listening in patients with situs inversus: brain asymmetry and situs asymmetry. Neuropsychologia 37:869–874.
Taylor DM. 1986. A bilateral gynandromorph of the Snow Crab, Chionoecetes-Opilio, from Newfoundland, Canada. Crustaceana 51:309–312.
Thitamadee S, Tuchihara K, Hashimoto T. 2002. Microtubule basis for left-handed helical growth in Arabidopsis. Nature 417:193–196.
Toga AW, Thompson PM. 2003. Mapping brain asymmetry. Nat Rev Neurosci 4:37–48.
Tsukui T, Capdevila J, Tamura K, Ruiz-Lozano P, Rodriguez-Esteban C, Yonei-Tamura S, Magallon J, Chandraratna RA, Chien K, Blumberg B, Evans RM, Belmonte JC. 1999. Multiple left-right asymmetry defects in Shh(-/-) mutant mice unveil a convergence of the shh and retinoic acid pathways in the control of Lefty-1. Proc Natl Acad Sci U S A 96: 11376–11381.
van Niekerk WA, Retief AE. 1981. The gonads of human true hermaphrodites. Hum Genet 58:117–122.
Vanhoven MK, Bauer Huang SL, Albin SD, Bargmann CI. 2006. The claudin superfamily protein nsy-4 biases lateral signaling to generate left-right asymmetry in C. elegans olfactory neurons. Neuron 51:291–302.
Vermot J, Pourquie O. 2005. Retinoic acid coordinates somitogenesis and left-right patterning in vertebrate embryos. Nature 435:215–220.
Vermot J, Gallego Llamas J, Fraulob V, Niederreither K, Chambon P, Dolle P. 2005. Retinoic acid controls the bilateral symmetry of somite formation in the mouse embryo. Science 308:563–566.
Viebahn C, Mayer B, Hrabe de Angelis M. 1995. Signs of the principle body axes prior to primitive streak formation in the rabbit embryo. Anat Embryol (Berl) 192: 159–169.
Ward HB, McLaren A, Baker TG. 1987. Gonadal development in T16H/XSxr hermaphrodite mice. J Reprod Fertil 81:295–300.
West PM, Love DR, Stapleton PM, Winship IM. 2003. Paternal uniparental disomy in monozygotic twins discordant for hemihypertrophy. J Med Genet 40:223–226.
Whitman M, Mercola M. 2001. TGF-beta superfamily signaling and left-right asymmetry. Science's STKE [Electronic Resource]: Signal Transduction Knowledge Environment 2001:RE1.
Wise SL, Meador KJ, Thompson WO, Avery SS, Loring DW, Wray BB. 1993. Cerebral lateralization and histamine skin test asymmetries in humans. Ann Allergy 70:328–332.
Xu J, Van Keymeulen A, Wakida NM, Carlton P, Berns MW, Bourne HR. 2007. Polarity reveals intrinsic cell chirality. Proc Natl Acad Sci U S A 104:9296–9300.
Zallen JA. 2007. Planar polarity and tissue morphogenesis. Cell 129:1051–1063.
Zhang M, Bolfing MF, Knowles HJ, Karnes H, Hackett BP. 2004. Foxj1 regulates asymmetric gene expression during left-right axis patterning in mice. Biochem Biophys Res Commun 324:1413–1420.
Zou EM, Fingerman M. 2000. External features of an intersex fiddler crab, Uca pugilator (Bosc, 1802) (Decapoda, Brachyura). Crustaceana 73:417–423.

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