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Perspectives and Open Problems in the Early Phases of Left-Right Patterning

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Summary

Embryonic left-right (LR) patterning is a fascinating aspect of embryogenesis. The field currently faces important questions about the origin of LR asymmetry, the mechanisms by which consistent asymmetry is imposed on the scale of the whole embryo, and the degree of conservation of early phases of LR patterning among model systems. Recent progress on planar cell polarity and cellular asymmetry in a variety of tissues and species provides a new perspective on the early phases of LR patterning. Despite the huge diversity in body-plans over which consistent LR asymmetry is imposed, and the apparent divergence in molecular pathways that underlie laterality, the data reveal conservation of physiological modules among phyla and a basic scheme of cellular chirality amplified by a planar cell polarity-like pathway over large cell fields.

Keywords

Left-right asymmetry; chirality; cytoskeleton; polarity; review

Introduction

The bilaterally-symmetric external body plan of vertebrates and many invertebrates conceals consistent left-right (LR) asymmetry in the shape and placement of internal organs. The striking conservation in the direction of asymmetry (e.g., consistency of the side on which the heart is located) is a remarkable aspect of embryonic development in a world in which no macroscopic force distinguishes left from right. LR patterning involves many of the key themes that fascinate developmental biologists: evolutionary mapping of a morphogenetic problem and molecular mechanisms upon different body-plans, a patterning event that cuts across multiple scales of organization, and a linkage among epigenetic, biophysical, and transcriptionally-mediated mechanisms (Levin 2006, Speder et al 2007b). Furthermore, errors of laterality have a significant clinical impact as birth defects (Ramsdell 2005).

Establishing consistent asymmetry requires three logical steps (Fig. 1). In the first phase of LR patterning, a mechanism must orient the LR axis with respect to the other two axes (Brown & Wolpert 1990), making one side different from the other. Defects in this process result in loss of asymmetry (midline heart, polysplenia or asplenia, etc.). The orientation of the LR axis must occur reliably with respect to the anterior-posterior and dorso-ventral axes so that all

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individuals are asymmetric in a consistent direction. Defects in this step result in a mixed population of offspring with mirror-image asymmetries (R- and L-handed forms). Downstream of this initial symmetry-breaking event lie asymmetric gene expression cascades; many aspects of this phase appear to be conserved in vertebrates including the asymmetric expression of members of the Nodal and Lefty families of TGF-β molecules and the transcription factor Pitx2 (Burdine & Schier 2000). The information needs to be amplified and transmitted to multiple organ systems, and midline structures must keep L-sided signals from affecting the R side and vice-versa. The final steps of organogenesis comprise a range of differential migration, proliferation, tension, and adhesion, as individual primordia utilize molecular cues to carry out distinct morphogenesis programs on either side of the midline. Lack of coordination causes a loss of coherence among organs' interpretation of LR information, resulting in heterotaxia (independently random placement of asymmetric structures among offspring).

It is now known that morphological asymmetry is determined by asymmetric gene expression on the L and R sides after gastrulation. However, despite exciting work on molecular mechanisms upstream of asymmetric transcription, the field still faces fundamental puzzles (Aw & Levin 2008, Tabin 2005). What is the first event that computes LR orientation? How are direction and position with respect to the midline communicated from putative LR organizers and imposed over distant cell fields? How conserved are the relevant molecular mechanisms throughout phyla? Here, we discuss several models based on novel recent findings (Aw et al 2008, Danilchik et al 2006, Takano et al 2007, Xu et al 2007).

Major Open Questions

1. What is the origin of asymmetry?

The classic treatment of asymmetry was put forth prior to the avalanche of molecular data produced more recently (Brown & Wolpert 1990). This lucid analysis postulated a chiral intracellular component (such as a chemical structure occurring as only one enantiomer in living cells) that was tethered or oriented with respect to the other two axes. This "F-molecule" could then nucleate directed intracellular transport in one consistent direction along the LR axis. This model is broadly compatible with many physical mechanisms and the basic scheme has held up remarkably well for the last 18 years.

Left-right asymmetry is a fundamental property of the Universe, revealed in parity violations during quantum decay (Wu et al 1957). Recent data indicate that chirality is derived within individual cells and may likewise be universal. One of the most significant findings for the field of LR asymmetry was recently made in the investigation of polarity in single neutrophil-like, unciliated HL60 cells that can orient the LR axis in culture (Xu et al 2007). Eighty eight percent of HL60 cells extended pseudopods to the left of a line connecting the nucleus and centrosome. Remarkably, these cells recapitulate all of the important phenotypes observed after manipulation of embryonic LR patterning, exhibiting isomerism, heterotaxia, and *situs inversus* after alteration of Par6, dynein, and GSK3β signals, respectively.

What subcellular component is responsible for the crucial orientation event that defines "leftward"? One likely possibility is that the coordination of the 3 axes is performed by a cytoskeletal organizing center such as the centriole or basal body. The early *Drosophila* embryo uses cytoskeletal nucleating centers to set up major body axes (Steinhauer & Kalderon 2006). The microtubule organizing center (MTOC) is an ideal and versatile candidate for an ancient, highly-conserved mechanism from which asymmetry can be leveraged. The MTOC nucleates microtubule assembly (Kellogg et al 1994) and can position itself in the exact center of even irregularly-shaped cells (McNiven & Porter 1988), suggesting it could act as the center of geometric computation to integrate the 3D morphology of the whole cell. In ciliates, basal bodies have a LR asymmetry that is linked to the overall chirality of the cell (Beisson & Jerka-

Dziadosz 1999, Bell et al 2008). Consistently, in human Bardet-Biedl patients, basal body dysfunction induces LR randomization (Ansley et al 2003). Recent work in a number of systems showed that cytoskeletal organization (Adams et al 2006, Qiu et al 2005, Thitamadee et al 2002) and the intracellular motors that move cargo molecules along those cytoskeletal tracks (Hozumi et al 2006, Nonaka et al 1998, Supp et al 1997) are all crucial for LR patterning. Indeed, in the cleaving frog egg, the cytoskeleton has a consistent bias that enables the asymmetric localization of physiological determinants of asymmetry (Aw et al 2008).

A recent study (Danilchik et al 2006) showed that the *Xenopus* egg cortex contains a maternally-derived cytoskeleton that has biased directionality. When the actin organization of the egg is disrupted pharmacologically, the animal pole rotates counter-clockwise with respect to the animal-vegetal axis, revealing the existence of consistent chirality in the egg. This suggests a model for how a cytoskeletal structure can derive a consistent direction along the LR axis from the dorso-ventral and anterior-posterior axes during fertilization and cleavage. The biased cytoskeleton, in an embryo with an inherent "North-South" axis (animal-vegetal, created by the maternal distribution of egg mRNAs and proteins) provides an "East-West" or counter-clockwise circumferential vector. Because consistent LR asymmetry (a true linear axis perpendicular to the other two) is set by the 2nd cleavage (Adams et al 2006), a mechanism is needed to convert the counter-clockwise cytoskeletal chirality of the egg into an orthogonal LR directionality in the embryo. The sperm entry point selects a unique place on the egg's circumference at which the counter-clockwise structure of the actin cytoskeleton provides an orthogonal LR arrow positioned perpendicular to the other two axes (Fig. 2A–A″).

2. What is the midline, and when is it determined?

Most discussions of asymmetry assume the existence of the midline. Behind this term are concealed several issues we do not understand. During organogenesis and later in development, the midline is defined as an imaginary line running from head to toe, across which the internal organs are normally placed asymmetrically. Earlier in development, i.e. during the period of asymmetric gene expression, the midline acts a barrier to prevent leak-over from side-specific signals (Bisgrove et al 1999). At this stage, the midline is what differentiates between directional information that is identical in all cells (which way is left-ward?), and positional information (am I on the R or L side of the midline?), which is different on the left and right sides of the embryo. The latter is required for side-specific gene expression. The midline also has considerable biomedical relevance (Martinez-Frias 1995); embryos that lack intact midline structures have laterality defects (Izraeli et al 1999, Przemeck et al 2003).

The problem is the lack of a molecular definition of the midline early in development, i.e. prior to asymmetric *Nodal* signaling. Thus, the early midline is currently just an imaginary line drawn through the middle of an organizer (expression of *Siamois* in frog, or base of the primitive streak in chick for example, Fig. 2B). It is unknown how the boundary between the L and R compartments of an embryonic field is determined. Data in *Xenopus* have provided one of the only glimpses into the mechanistic, molecular definition of the L:R boundary. In the 16-cell frog embryo, the L and R ventral cells are gap-junctionally isolated (Guthrie 1984); this zone of isolation splits an otherwise gap junction-coupled field and demarcates the midline of the early embryo. Targeted disruption of the ventral isolation zone via misexpression of constitutively open gap junctions results in specific randomization of asymmetry (Levin & Mercola 1998), confirming functionally the significance of the midline established by lack of gap junctional communication between cells separated at the very first cleavage.

This mechanism relies on the fact that in *Xenopus*, the cleavage plane of the first large blastomeres normally coincides with the prospective midline of the whole embryo such that the left side of each cell also corresponds with the left side of the embryo (Fig. 2B′). What happens in embryos that establish the LR axis when there are thousands of cells present that

do not span the whole embryo and when intracellular transport in individual cells cannot distribute components across the entire embryo? Pharmacological or molecular disruption of gap junctional communication in chick (where the midline is not apparent until there are thousands of cells) also randomizes asymmetric gene expression (Levin & Mercola 1999). However, it is not known whether the observed pattern of junctional isolation throughout the streak (Levin & Mercola 1999) is involved in the generation of a sharp midline within the streak field.

When does the midline really appear? It is thought that the radial symmetry of the avian blastoderm is broken by a streak-inducing center and that the midline, and thus LR asymmetry, is elaborated during gastrulation. While an axis can be experimentally induced at later stages in mice and birds, there is the possibility that the midline, and perhaps asymmetry, is set up in these organisms much earlier.

Gynandromorphs display both male and female characteristics due to non-disjunction of sex chromosomes during early cell divisions. Bilateral gynandromorphs provide a striking visible demarcation of the midline in organisms where the male and female are sexually dimorphic, typically because of coloring differences (Fig. 2C–C″). Gynandromorphism occurs in a wide variety of species including insects (most commonly reported in butterflies), crabs, chickens (reviewed in (Levin 2006)), and even human patients with X-linked diseases such as CHILD syndrome (Happle 2006). The boundary is often exactly down the middle, including in the brain (Agate et al 2003), and in roosters, is respected even by the comb on top of the head of the male half.

The sharp midline separation suggests that the first cell cleavage may produce L and R halves that inherit differential chiral information. Is this plausible in mammals? The main axes of rabbit embryos are known to be established prior to the appearance of the streak (Viebahn et al 1995) and this has been proposed for mouse development (Gardner 2001, Piotrowska & Zernicka-Goetz 2001). Even in humans, unilateral X-inactivation required to explain the harlequin pattern observed in CHILD syndrome (Happle 2002) requires that the midline already exist at very early stages. This would be consistent with the puzzling "mirroring" (opposite sidedness of unilateral traits) often observed in human monozygotic twins (reviewed in (Levin 1999)).

3. What mechanisms are upstream of asymmetric gene expression?

Ultimately, early pathways must initiate differential gene expression cascades on the L and R sides. There are at least two types of biophysical mechanisms functioning upstream of asymmetric transcription.

Elegant studies examining movement of cilia show that their beating generates a biased fluid flow (Kramer-Zucker et al 2005, Nonaka et al 2002, Schweickert et al 2007) that could result in asymmetric extracellular signals. While cilia are commonly viewed as an initiator of asymmetry, we have argued elsewhere that no data indicate that cilia initiate asymmetry; much of the genetic data are also consistent with intracellular transport roles for ciliary proteins (Levin 2003). We view the cytoskeleton-dependent events described above as more fundamental and broadly relevant to numerous species where asymmetry is established without cilia or long prior to the appearance of cilia (Levin & Palmer 2007, Speder et al 2007a). Additionally, ciliary function is not likely a general LR initiating step because their function is not upstream of some LR pathways such as asymmetric *Notch* signals (Krebs et al 2003, Raya et al 2003), and because human patients with primary ciliary diskinesia display normal handedness and brain laterality (McManus et al 2004). Ciliary beating may instead be a middle step that amplifies or converts earlier signals.

Further analysis of mutants such as the zebrafish *Seahorse* (Kishimoto et al 2008) is likely to shed light on this issue. Seahorse genetically interacts with the ciliary gene Inversin, is highly enriched in heavily ciliated tissues, and is required for normal asymmetry and for preventing kidney cyst formation. However, the LR randomization in *Seahorse* mutants occurs in the absence of detectable ciliary motility or structure defects. This illustrates the dissociation between associated kidney/laterality syndromes, usually thought to be due to ciliary defects, from ciliary motility *per se* and supports models of non-ciliary roles for "ciliary" proteins in LR patterning. Indeed, *Seahorse* contains nuclear import/export sequences; its precise function remains to be characterized.

Another epigenetic system functioning upstream of asymmetric gene expression is bioelectrical. The LR-differential function of specific ion transporters, and their resulting voltage and pH gradients, are required for normal asymmetry at early stages of sea urchin, *Ciona*, chick, frog, and zebrafish embryonic development (reviewed in (Levin 2006)). The details are clearest in *Xenopus*, where intracellular localization via a biased cytoskeleton during the first cleavages result in LR-asymmetric distribution of maternal ion transporter proteins. Just as in the mammalian gut (Kaufhold et al 2008), the module consists of the Kir4.1 and KCNQ1 channels and the H^+/K^+-ATPase exchanger (Aw et al 2008, Levin et al 2002). Together with the V-ATPase H^+ pump (Adams et al 2006), this results in the right side of the early embryo being more strongly polarized than the left – a state that is absolutely required for normal subsequent asymmetric gene expression. In the chick, the proximal sensor is *Notch*, which is activated via a Ca^{++}-dependent pathway (Raya et al 2004).

4. How is subcellular chirality imposed on large cell fields?

It is likely that LR information originates intracellularly. This may occur in all cells (if the orientation mechanism is extremely ancient and fundamental, as suggested by data from ciliates and HL60 cells) or perhaps only in a LR "organizer" (Hyatt & Yost 1998, Nascone & Mercola 1997). In either case, intracellular information must be amplified and coherently imposed across large cell fields; this process must interact with the conversion of directional to positional information along the LR axis.

One possibility is the creation of a morphogen gradient by ciliary flow (Tanaka et al 2005). Another is the electrophoretic movement of a LR determinant. In chick and frog, the neurotransmitter serotonin plays a key role in LR patterning upstream of asymmetric gene expression and long before the appearance of neurons and cilia (Fukumoto et al 2005). While a closely related pathway also functions in the LR patterning of plants (Pekker et al 2005), the details of serotonin's movement in chick and other species are unclear. In *Xenopus* however, the system has been worked out in considerable detail (Levin et al 2006); maternal serotonin appears to accumulate in a right-ward gradient through a path of gap junction-coupled cells under the electrophoretic force of the asymmetric voltage gradient. Because the relevant parameters are now known quantitatively, a predictive mechanistic model has been formulated of this process (Esser et al 2006). By creating a voltage gradient, a subcellular asymmetric localization of ion transporters is transduced into a multicellular morphogen gradient that provides LR positional information across the entire embryonic field.

One other pathway now emerges as a fascinating candidate for a well-conserved role in amplifying LR pattern: planar cell polarity (PCP) - a powerful, elegant, and widely conserved module for imposing a coordinated orientation on a field of cells (Fig. 2D,D′). PCP is the coordinated polarization of cells in the plane of an epithelium, which occurs in the *Drosophila* wing bristles and the sensory hair cells of the vertebrate ear (Wang & Nathans 2007). PCP must solve the very same issues that confront LR asymmetry (Lewis & Davies 2002), and a logical schematic of PCP maps directly onto the F-molecule model of LR patterning. How is the internal asymmetry of the cell generated? How is the orientation of the

axis of asymmetry (the polarity vector) of a given cell coupled to that of its neighbors? And, how is the orientation of a tissue controlled globally by influences from more distant structures, according to its location in the body as a whole? Through a process akin to domineering nonautonomy (Amonlirdviman et al 2005), LR information arising in a small group of cells can thus be amplified across large cell fields in a coordinated manner.

The PCP model requires that embryos at relevant stages propagate LR information through polarized epithelia. The early-cleavage frog embryo is an epithelium (Muller & Hausen 1995) and it was recently shown (Aw et al 2008) that the 4-cell embryo integrates the orthogonal apical-basal (animal-vegetal) and planar (DV and LR) polarities to orient intracellular transport tracks that are biased towards the right-ventral cell. The early chick blastoderm is also an epithelium that is polarized in the plane. The cells have a clear dorso-ventral (apical-basal) polarity, and reveal their knowledge of anterior-posterior polarity by their alignment during streak elongation (Wei & Mikawa 2000), a key process now known to be dependent on the PCP pathway (Voiculescu et al 2007). During stages at which the first known LR asymmetric signal – the left-sided depolarization – appears (Levin et al 2002), the chick blastoderm, like Drosophila, exhibits features dependent on core PCP proteins, including characteristic multicellular rosettes (Blankenship et al 2006, Wagstaff et al 2008), intercalation (Benazeraf & Pourquie 2008), and convergent extension, a process of coordinated, polarized movement of a field of cells (Lawson & Schoenwolf 2001).

Both PCP and LR asymmetry involve conserved, widespread systems for orienting a large-scale third axis in relation to two existing axes. This provides a ready framework to understand the way that subcellular chirality is imposed on a complete body axis and test models in which chiral molecules (Brown & Wolpert 1990) nucleate asymmetry in organisms that pattern the LR axis at later stages, within multicellular fields of different sizes. The origin of LR information in chick is mysterious because asymmetry appears when the blastoderm contains thousand of small cells, which unlike *Xenopus* blastomeres do not have the opportunity to utilize intracellular transport machinery to localize protein cargo to the L or R sides of the prospective embryonic midline. One of the most attractive features of PCP for understanding LR patterning throughout phyla is its scale invariance. By setting up alignment of polarization on the cellular level, PCP mechanisms allow embryos to achieve large-scale polarization from intracellular events.

5. How conserved are early LR mechanisms?

We have argued for considerable conservation of basic LR mechanisms (Levin 2006) and suggest widespread reuse of a module consisting of intracellular chirality driven by cytoskeletal organizing centers and amplification of signals through subsequent physiological asymmetries. This proposal is controversial, with many in the field favoring independently-evolved mechanisms (Tabin 2005). A meta-analysis of molecular mechanisms that have been probed in LR patterning (Fig. 3 and (Levin & Palmer 2007)) suggests that while the details differ, many mechanisms are broadly conserved.

More importantly, what may be conserved are the higher-level modules performing functions such as orientation, amplification, and conversion of direction to position with respect to the midline. If the information processing by each of these modules remains intact, the molecular details of each (i.e. the specific proteins involved) can diverge, as can the timing of each step relative to other developmental events. For example, *Xenopus* uses ion currents to amplify asymmetry at the first few cleavages, while the chick waits until there are ~50K cells. Could this be an example of heterochrony, where different species conserve the same basic steps but change the timing of the LR pathway's mechanisms to better match their architecture?

6. What's next? Puzzles and directions

One of the issues facing the field is that some important and fascinating phenomena are not easy to study in the popular "genetic" model systems. Examples include bilateral gynandromorphs in birds and most insects, and opposite-sided hair whorls in monozygotic human twins. The lack of midline separation of male and female tissues in *Drosophila* gynandromorphs (unlike moths and butterflies), the random separation of male-female cells in the CHILD syndrome mouse model (unlike the strict midline demarcation in humans), and the lack of data on early, pre-streak physiological LR mechanisms in the highly unusual cylinder of the rodent embryo suggest that atypical features of some model species may lead us astray when looking for general mechanisms. Testing for conservation of LR mechanisms (e.g., gap junctional communication) is further complicated by the importance of genetic background: mice with conditional deletion of the gap junction protein *Connexin43* exhibited disruption of neuronal migration ranging from no phenotype in C57B1/6J mice to a strong phenotype in 129SVEV background (Wiencken-Barger et al 2007).

The PCP hypothesis predicts a link between the planar polarity of epithelia and LR patterning. In this model, the known role of planar polarity in determining hair whorl patterns (Guo et al 2004, Wang et al 2006) explains the mysterious relationship between the direction of hair whorls on the scalp and laterality of the brain and body in monozygotic twins (Jansen et al 2007, Levin 1999, Weber et al 2006). Interestingly, genetic deletion of the PCP gene *Frizzled6* results in ectopic hair whorls where straight hair patterns normally occur (Guo et al 2004). Future work will reveal whether PCP is necessary to convert a rotary pattern into a straight one (a multi-cellular version of Fig. 2A′) and whether PCP is necessary to mask the chirality inherent in structures, the way that the retinoic acid pathway does in somite patterning (Vermot & Pourquie 2005).

Another major theme for future studies of LR patterning is likely to be epigenetics. For example, the asymmetry of the asexual blastozooid of ascidians appears to not be controlled by the genome (described in (Ishii et al 1994)). Additionally, both ciliary and physiological modules' signaling does not require changes in transcription. It is crucial to understand the linkage of these pathways to canonical response cascades. We predict that interactions will be detected between canonical planar polarity proteins and H^+ pumps and K^+ channels, in both vertebrate and invertebrate models of LR asymmetry; testing this hypothesis is an important future direction.

Models in which the chiral computation occurs soon after fertilization imply that twins produced by blastomere separation will exhibit reversals of asymmetry, whereas models where LR asymmetry is initiated by cilia in later embryogenesis suggest that early-split twins should both be normal since the cilia will beat normally in each twin at gastrulation. The conservation of chirality within a pair of cells derived from the same mother cell is beautifully illustrated by the work of Albrecht-Buehler, who showed that after division, daughters of mammalian cells in culture have mirror-image cytoskeletal organization and subsequent migration trajectories (Albrecht-Buehler 1977). The medical literature contains numerous and as yet unexplained examples of such mirroring (see (Levin 1999, Levin 2001)), including opposite hair-whorl direction and unilateral facial defects in monozygotic twins. When separated at the 2-cell stage, Newt embryos exhibit 89% incidence of organ laterality reversal in one of the twins (Takano et al 2007). Thus, the twinning data do not support ciliary models but are a straightforward prediction of asymmetry generation by very early, intracellular chirality mechanisms. Because some bovine strains have hair whorls, future studies using *in vitro* fertilization could be used to generate monozygotic twins with known timing of cell separation, which can then be examined for hair whorl direction.

Taken together, we believe the data argue for a view of LR patterning as an instance of universal, ancient cellular polarity that originates inside the cell, magnified by functionally-conserved physiological and planar coordination modules. The establishment of new model systems will advance major open directions for the field, including the nature of the midline, the molecular mechanisms underlying organ concordance, and subtle asymmetries such as handedness, hair whorl chirality, and brain laterality. Quantitative modeling at multiple levels of organization, together with molecular investigation of LR signaling components, is likely to reveal fascinating new aspects of developmental and cell biology.

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References

Adams DS, Robinson KR, Fukumoto T, Yuan S, Albertson RC, et al. Early, H+-V-ATPase-dependent proton flux is necessary for consistent left-right patterning of non-mammalian vertebrates. Development 2006;133:1657–71. [PubMed: 16554361]

Agate RJ, Grisham W, Wade J, Mann S, Wingfield J, et al. Neural, not gonadal, origin of brain sex differences in a gynandromorphic finch. Proc Natl Acad Sci U S A 2003;100:4873–8. [PubMed: 12672961]

Albrecht-Buehler G. Daughter 3T3 cells. Are they mirror images of each other? J Cell Biol 1977;72:595–603. [PubMed: 838769]

Amonlirdviman K, Khare NA, Tree DR, Chen WS, Axelrod JD, Tomlin CJ. Mathematical modeling of planar cell polarity to understand domineering nonautonomy. Science 2005;307:423–6. [PubMed: 15662015]

Ansley SJ, Badano JL, Blacque OE, Hill J, Hoskins BE, et al. Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome. Nature 2003;425:628–33. [PubMed: 14520415]

Aw S, Adams DS, Qiu D, Levin M. H,K-ATPase protein localization and Kir4.1 function reveal concordance of three axes during early determination of left-right asymmetry. Mech Dev 2008;125:353–72. [PubMed: 18160269]

Aw S, Levin M. What's left in asymmetry? Dev Dyn. 2008

Beisson J, Jerka-Dziadosz M. Polarities of the centriolar structure: morphogenetic consequences. Biol Cell 1999;91:367–78. [PubMed: 11419478]

Bell AJ, Satir P, Grimes GW. Mirror-imaged doublets of Tetmemena pustulata: implications for the development of left-right asymmetry. Dev Biol 2008;314:150–60. [PubMed: 18164285]

Benazeraf B, Pourquie O. Developmental biology: cell intercalation one step beyond. Curr Biol 2008;18:R119–21. [PubMed: 18269906]

Bisgrove BW, Essner JJ, Yost HJ. Regulation of midline development by antagonism of lefty and nodal signaling. Development 1999;126:3253–62. [PubMed: 10375514]

Blankenship JT, Backovic ST, Sanny JS, Weitz O, Zallen JA. Multicellular rosette formation links planar cell polarity to tissue morphogenesis. Dev Cell 2006;11:459–70. [PubMed: 17011486]

Brown NA, Wolpert L. The development of handedness in left/right asymmetry. Development 1990;109:1–9. [PubMed: 2209459]

Burdine R, Schier A. Conserved and divergent mechanisms in left-right axis formation. Genes & Development 2000;14:763–76. [PubMed: 10766733]

Danilchik MV, Brown EE, Riegert K. Intrinsic chiral properties of the Xenopus egg cortex: an early indicator of left-right asymmetry? Development 2006;133:4517–26. [PubMed: 17050623]

Esser AT, Smith KC, Weaver JC, Levin M. Mathematical model of morphogen electrophoresis through gap junctions. Dev Dyn 2006;235:2144–59. [PubMed: 16786594]

Fukumoto T, Kema IP, Levin M. Serotonin signaling is a very early step in patterning of the left-right axis in chick and frog embryos. Curr Biol 2005;15:794–803. [PubMed: 15886096]

Gardner RL. Specification of embryonic axes begins before cleavage in normal mouse development. Development - Supplement 2001;128:839–47.

Guo N, Hawkins C, Nathans J. Frizzled6 controls hair patterning in mice. Proc Natl Acad Sci U S A 2004;101:9277–81. [PubMed: 15169958]

Guthrie S. Patterns of junctional communication in the early amphibian embryo. Nature 1984;311:149–51. [PubMed: 6206399]

Happle R. Dohi Memorial Lecture. New aspects of cutaneous mosaicism. J Dermatol 2002;29:681–92. [PubMed: 12484429]

Happle R. X-chromosome inactivation: role in skin disease expression. Acta Paediatricia Suppl 2006;95:9–10.

Hozumi S, Maeda R, Taniguchi K, Kanai M, Shirakabe S, et al. An unconventional myosin in Drosophila reverses the default handedness in visceral organs. Nature 2006;440:798–802. [PubMed: 16598258]

Hyatt BA, Yost HJ. The left-right coordinator: the role of Vg1 in organizing left-right axis formation. Cell 1998;93:37–46. [PubMed: 9546390]

Ishii T, Taneda Y, Saito Y. Polarity and Body Pattern in Ascidian Chimeras Formed between Normal (Situs Normale Viscerum) and Reversed (Situs-Inversus-Viscerum) Bodies. Journal of Experimental Zoology 1994;269:336–48.

Izraeli S, Lowe LA, Bertness VL, Good DJ, Dorward DW, et al. The SIL gene is required for mouse embryonic axial development and left-right specification. Nature 1999;399:691–4. [PubMed: 10385121]

Jansen A, Lohmann H, Scharfe S, Sehlmeyer C, Deppe M, Knecht S. The association between scalp hair-whorl direction, handedness and hemispheric language dominance: is there a common genetic basis of lateralization? Neuroimage 2007;35:853–61. [PubMed: 17275334]

Kaufhold MA, Krabbenhoft A, Song P, Engelhardt R, Riederer B, et al. Localization, trafficking, and significance for acid secretion of parietal cell Kir4.1 and KCNQ1 K+ channels. Gastroenterology 2008;134:1058–69. [PubMed: 18395087]

Kellogg DR, Moritz M, Alberts BM. The centrosome and cellular organization. Annu Rev Biochem 1994;63:639–74. [PubMed: 7979251]

Kishimoto N, Cao Y, Park A, Sun Z. Cystic kidney gene seahorse regulates cilia-mediated processes and Wnt pathways. Dev Cell 2008;14:954–61. [PubMed: 18539122]

Konig A, Happle R, Bornholdt D, Engel H, Grzeschik KH. Mutations in the NSDHL gene, encoding a 3beta-hydroxysteroid dehydrogenase, cause CHILD syndrome. Am J Med Genet 2000;90:339–46. [PubMed: 10710235]

Kramer-Zucker AG, Olale F, Haycraft CJ, Yoder BK, Schier AF, Drummond IA. Cilia-driven fluid flow in the zebrafish pronephros, brain and Kupffer's vesicle is required for normal organogenesis. Development 2005;132:1907–21. [PubMed: 15790966]

Krebs LT, Iwai N, Nonaka S, Welsh IC, Lan Y, et al. Notch signaling regulates left-right asymmetry determination by inducing Nodal expression. Genes Dev 2003;17:1207–12. [PubMed: 12730124]

Lawson A, Schoenwolf GC. New insights into critical events of avian gastrulation. Anat Rec 2001;262:238–52. [PubMed: 11241193]

Levin M. Twinning and embryonic left-right asymmetry. Laterality 1999;4:197–208. [PubMed: 15513114]

Levin, M. Asymmetry of Body and Brain: Embryological and Twin Studies. In: Smelser, NBP., editor. International Encyclopedia of Social and Behavioral Sciences. Oxford, UK: Elsevier; 2001. p. 853-9.

Levin M. Motor protein control of ion flux is an early step in embryonic left-right asymmetry. Bioessays 2003;25:1002–10. [PubMed: 14505367]

Levin M. 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 2006;78:191–223. [PubMed: 17061264]

Levin M, Buznikov GA, Lauder JM. Of minds and embryos: left-right asymmetry and the serotonergic controls of pre-neural morphogenesis. Dev Neurosci 2006;28:171–85. [PubMed: 16679764]

Levin M, Mercola M. Gap junctions are involved in the early generation of left-right asymmetry. Dev Biol 1998;203:90–105. [PubMed: 9806775]

Levin M, Mercola M. Gap junction-mediated transfer of left-right patterning signals in the early chick blastoderm is upstream of Shh asymmetry in the node. Development 1999;126:4703–14. [PubMed: 10518488]

Levin M, Palmer AR. Left-right patterning from the inside out: widespread evidence for intracellular control. Bioessays 2007;29:271–87. [PubMed: 17295291]

Levin M, Thorlin T, Robinson KR, Nogi T, Mercola M. Asymmetries in H+/K+-ATPase and cell membrane potentials comprise a very early step in left-right patterning. Cell 2002;111:77–89. [PubMed: 12372302]

Lewis J, Davies A. Planar cell polarity in the inner ear: how do hair cells acquire their oriented structure? J Neurobiol 2002;53:190–201. [PubMed: 12382275]

Martinez-Frias ML. Primary midline developmental field. I. Clinical and epidemiological characteristics. Am J Med Genet 1995;56:374–81. [PubMed: 7604845]

McManus IC, Martin N, Stubbings GF, Chung EM, Mitchison HM. Handedness and situs inversus in primary ciliary dyskinesia. Proc R Soc Lond B Biol Sci 2004;271:2579–82.

McNiven MA, Porter KR. Organization of microtubules in centrosome-free cytoplasm. J Cell Biol 1988;106:1593–605. [PubMed: 3286659]

Moeller H, Jenny A, Schaeffer HJ, Schwarz-Romond T, Mlodzik M, et al. Diversin regulates heart formation and gastrulation movements in development. Proc Natl Acad Sci U S A 2006;103:15900–5. [PubMed: 17032765]

Muller HA, Hausen P. Epithelial cell polarity in early Xenopus development. Dev Dyn 1995;202:405–20. [PubMed: 7626797]

Nascone N, Mercola M. Organizer induction determines left-right asymmetry in Xenopus. Dev Biol 1997;189:68–78. [PubMed: 9281338]

Nonaka S, Shiratori H, Saijoh H, Hamada H. Determination of left-right patterning of the mouse embryo by artificial nodal flow. Nature 2002;418:96–9. [PubMed: 12097914]

Nonaka S, Tanaka Y, Okada Y, Takeda S, Harada A, et al. Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. Cell 1998;95:829–37. [PubMed: 9865700]

Pekker I, Alvarez JP, Eshed Y. Auxin response factors mediate Arabidopsis organ asymmetry via modulation of KANADI activity. Plant Cell 2005;17:2899–910. [PubMed: 16199616]

Piotrowska K, Zernicka-Goetz M. Role for sperm in spatial patterning of the early mouse embryo. Nature 2001;409:517–21. [PubMed: 11206548]

Przemeck GKH, Heinzmann U, Beckers J, de Angelis MH. Node and midline defects are associated with left-right development in Delta1 mutant embryos. Development 2003;130:3–13. [PubMed: 12441287]

Qiu D, Cheng SM, Wozniak L, McSweeney M, Perrone E, Levin M. Localization and loss-of-function implicates ciliary proteins in early, cytoplasmic roles in left-right asymmetry. Dev Dyn 2005;234:176–89. [PubMed: 16059906]

Ramsdell AF. Left-right asymmetry and congenital cardiac defects: getting to the heart of the matter in vertebrate left-right axis determination. Dev Biol 2005;288:1–20. [PubMed: 16289136]

Raya A, Kawakami Y, Rodriguez-Esteban C, Buscher D, Koth CM, et al. Notch activity induces Nodal expression and mediates the establishment of left-right asymmetry in vertebrate embryos. Genes Dev 2003;17:1213–8. [PubMed: 12730123]

Raya A, Kawakami Y, Rodriguez-Esteban C, Ibanes M, Rasskin-Gutman D, et al. Notch activity acts as a sensor for extracellular calcium during vertebrate left-right determination. Nature 2004;427:121–8. [PubMed: 14712268]

Schweickert A, Weber T, Beyer T, Vick P, Bogusch S, et al. Cilia-driven leftward flow determines laterality in Xenopus. Curr Biol 2007;17:60–6. [PubMed: 17208188]

Speder P, Petzoldt A, Suzanne M, Noselli S. Strategies to establish left/right asymmetry in vertebrates and invertebrates. Curr Opin Genet Dev. 2007a

Speder P, Petzoldt AG, Suzanne M, Noselli S. Strategies to establish left/right asymmetry in vertebrates and invertebrates. Current Opinion in Genetics & Development 2007b;17:351–8. [PubMed: 17643981]

Steinhauer J, Kalderon D. Microtubule polarity and axis formation in the Drosophila oocyte. Dev Dyn 2006;235:1455–68. [PubMed: 16586443]

Supp DM, Witte DP, Potter SS, Brueckner M. Mutation of an axonemal dynein affects left-right asymmetry in inversus viscerum mice. Nature 1997;389:963–6. [PubMed: 9353118]

Tabin C. Do we know anything about how left-right asymmetry is first established in the vertebrate embryo? J Mol Histol 2005:1–7. [PubMed: 15703994]

Takano K, Ito Y, Obata S, Oinuma T, Komazaki S, et al. Heart formation and left-right asymmetry in separated right and left embryos of a newt. Int J Dev Biol 2007;51:265–72. [PubMed: 17554678]

Tanaka Y, Okada Y, Hirokawa N. FGF-induced vesicular release of Sonic hedgehog and retinoic acid in leftward nodal flow is critical for left-right determination. Nature 2005;435:172–7. [PubMed: 15889083]

Thitamadee S, Tuchihara K, Hashimoto T. Microtubule basis for left-handed helical growth in *Arabidopsis*. Nature 2002;417:193–6. [PubMed: 12000963]

Vermot J, Pourquie O. Retinoic acid coordinates somitogenesis and left-right patterning in vertebrate embryos. Nature 2005;435:215–20. [PubMed: 15889094]

Viebahn C, Mayer B, Hrabe de Angelis M. Signs of the principle body axes prior to primitive streak formation in the rabbit embryo. Anat Embryol (Berl) 1995;192:159–69. [PubMed: 7486012]

Voiculescu O, Bertocchini F, Wolpert L, Keller RE, Stern CD. The amniote primitive streak is defined by epithelial cell intercalation before gastrulation. Nature 2007;449:1049–52. [PubMed: 17928866]

Wagstaff LJ, Bellett G, Mogensen MM, Munsterberg A. Multicellular rosette formation during cell ingression in the avian primitive streak. Dev Dyn 2008;237:91–6. [PubMed: 18069691]

Wang Y, Badea T, Nathans J. Order from disorder: Self-organization in mammalian hair patterning. Proc Natl Acad Sci U S A 2006;103:19800–5. [PubMed: 17172440]

Wang Y, Nathans J. Tissue/planar cell polarity in vertebrates: new insights and new questions. Development 2007;134:647–58. [PubMed: 17259302]

Weber B, Hoppe C, Faber J, Axmacher N, Fliessbach K, et al. Association between scalp hair-whorl direction and hemispheric language dominance. Neuroimage 2006;30:539–43. [PubMed: 16289721]

Wei Y, Mikawa T. Formation of the avian primitive streak from spatially restricted blastoderm: evidence for polarized cell division in the elongating streak. Development 2000;127:87–96. [PubMed: 10654603]

Wiencken-Barger AE, Djukic B, Casper KB, McCarthy KD. A role for Connexin43 during neurodevelopment. Glia 2007;55:675–86. [PubMed: 17311295]

Wu C, Ambler E, Hayward R, Hoppes D, Hudson R. Experimental test of parity conservation in beta decay. Physical review 1957;105:1413.

Xu J, Van Keymeulen A, Wakida NM, Carlton P, Berns MW, Bourne HR. Polarity reveals intrinsic cell chirality. Proc Natl Acad Sci U S A 2007;104:9296–300. [PubMed: 17517645]

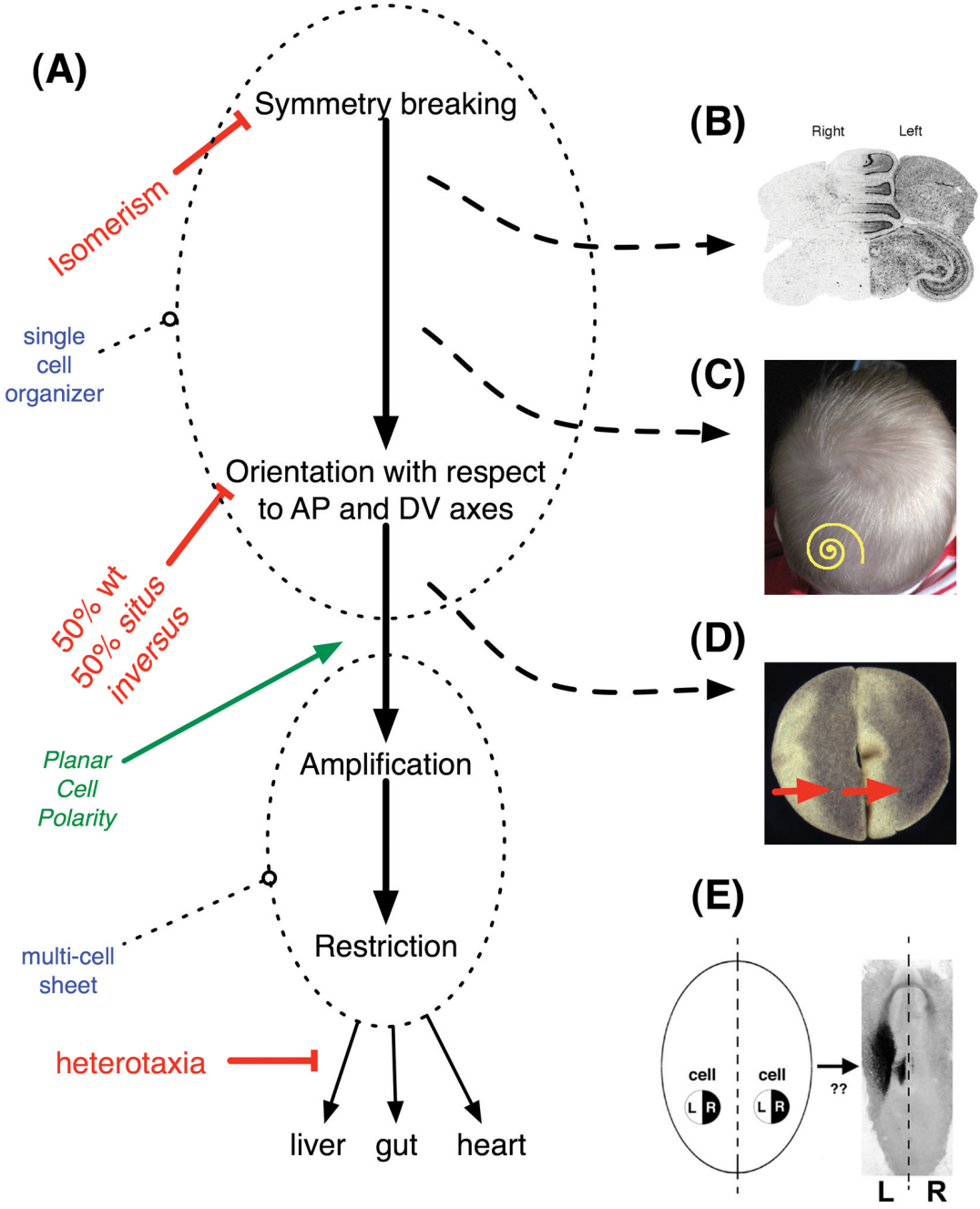

Figure 1.

Conceptual phases of LR patterning and their later readouts

(**A**) LR symmetry breaking requires that a midline be established, and one side be made different from the other. This difference needs to be consistently oriented within the population. The information needs to be amplified and transmitted to multiple organ systems; midline structures must perform a restriction function to side-specific signals from crossing over. Lack of coordination results in heterotaxia, where organs make independent decisions resulting in a spectrum of random placement. Red arrows indicate phenotypes arising from disruption of each step.

(**B**) *In situ* hybridization of a brain section from a songbird in which chromosomal aberrations during the first cleavages resulted in a gynandromorphy (chimera of male and female cells). The division between the female chromosome cells (dark signal) and the male chromosome cells (no signal) is precisely down the anatomical midline of the brain, suggesting that the embryonic midline is determined long prior to streak development in bird in development. Taken from Fig. 6 of (Agate et al 2003), copyright held by National Academy of Sciences.
(**C**) Hair whorls indicate the presence of chirality distinct from the *situs* of the body organs. In monozygotic twins, such hair whorls are mirror images, revealing that splitting of mammalian embryos results in subtle asymmetries initiated very early and not reversed by later embryonic events.
(**D**) Immunohistochemistry of a 2-cell frog embryo reveals the first step of a polarization process, where an intracellular protein is localized to one side of each cell (revealing direction and subcellular planar polarity along the LR axis, red arrowheads).
(**E**) During the orientation and amplification phases, cells must convert intracellular knowledge of direction along the LR axis (the same in all cells) into position relative to the midline (different in L vs. R cells), here illustrated by the expression of *Nodal* in lateral plate cells only on the left side of the chick embryo.

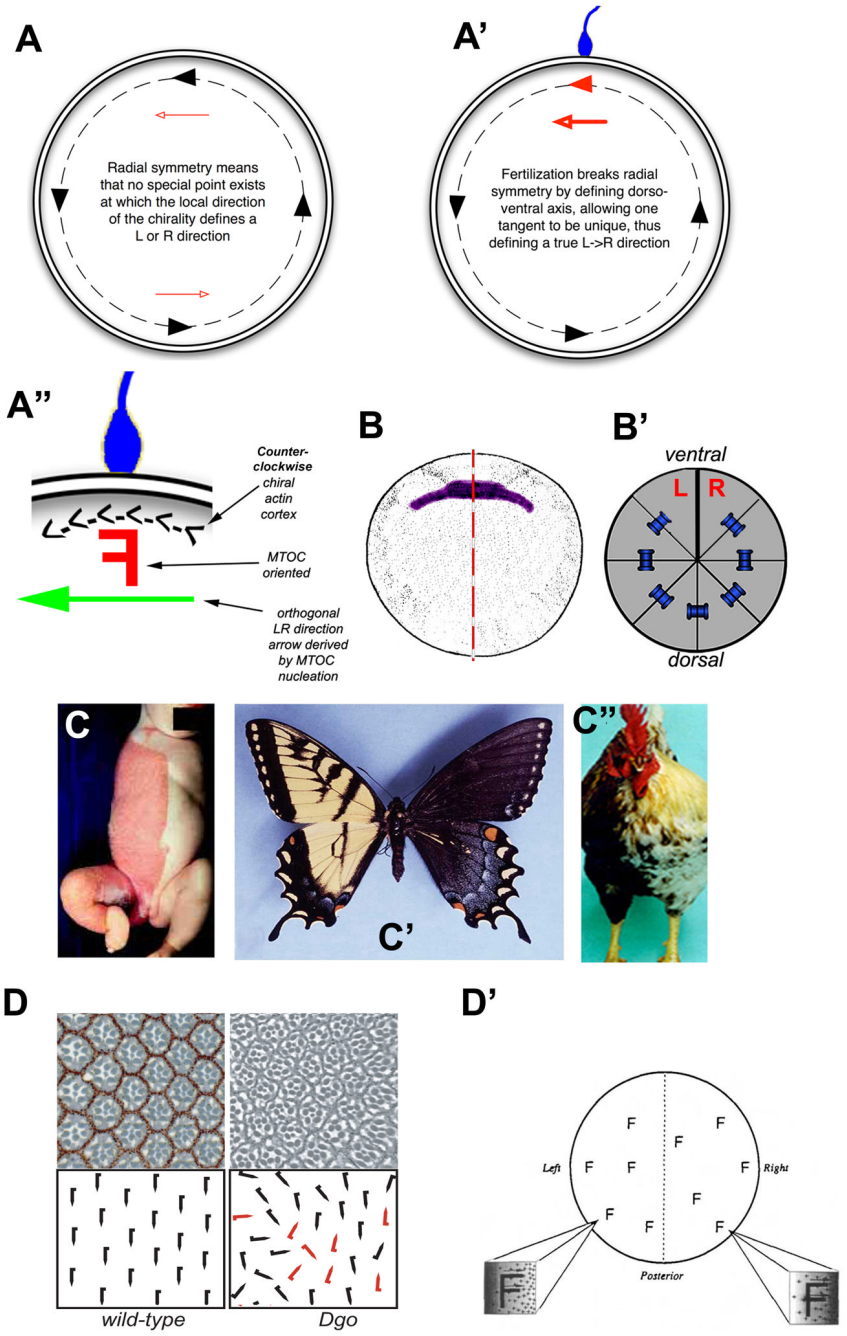

Figure 2.

Elaboration of asymmetry: from single cells to cell fields

Consistent "East-West" (counterclockwise) chirality (Danilchik et al 2006) exists in the actin cytoskeleton around the periphery of the *Xenopus* egg (black dashed line; the egg is viewed from the animal pole with the animal-vegetal axis perpendicular to the plane of the page). (**A**) This provides different LR directional cues at distinct tangent points along the periphery (black arrowheads along the dashed line) and offers no unique LR orientation because each point on the circumference is equivalent to the others (red arrowheads show that this cue points right-ward on one side and left-ward on the opposite side). (**A′**) At fertilization, sperm entry breaks the radial symmetry and determines a specific point on the circumference through which

the midline axis of bilateral symmetry passes. The chiral orientation of the actin cytoskeleton at that unique point converts the bilateral symmetry into LR asymmetry through a linear cue along the LR axis (red arrowhead at the tangent point defined by the sperm entry). This shows how circumferential chirality (East-West) can be converted into an organism-wide linear directionality (Left-Right) once the dorsoventral axis is determined. Close-up is in (**A**″), where a putative chiral "F-molecule" in the MTOC can be oriented with respect to the actin cortex and nucleate microtubule transport paths that have a true LR directionality.

(**B**) *Goosecoid* expression designates the frog organizer; this identifies the future dorsal side, but the midline is an imaginary line drawn through the middle of this region and it is unclear how it is determined in most species. (**B**′) In *Xenopus*, the midline is defined by the lack of gap junctional communication between the L and R ventral cells (blue cylinders represent gap junctions).

(**C**) Asymmetric cutaneous pigmentation pattern with a sharp midplane demarcation in the X-linked CHILD syndrome of humans (reproduced with permission from John Wiley and Sons, from (Konig et al 2000)). (**C**′) A gynandromorphic swallowtail butterfly, *Papilio glaucas* (dorsal view; brightly-colored left side of body is male; courtesy of James Adams). (**C**″) Gynandromorph chicken (courtesy of Michael Clinton).

(**E**) Coherence of consistent orientation of eye cells in *Drosophila* is disrupted after overexpression of the planar cell polarity protein *Diego*. Taken with permission from (Moeller et al 2006). (**E**″) This system shares remarkable similarity with the F-molecule model of (Brown & Wolpert 1990), where plane-polarized elements coupled to directed transport allow intracellular chirality to impose asymmetry across cell fields.

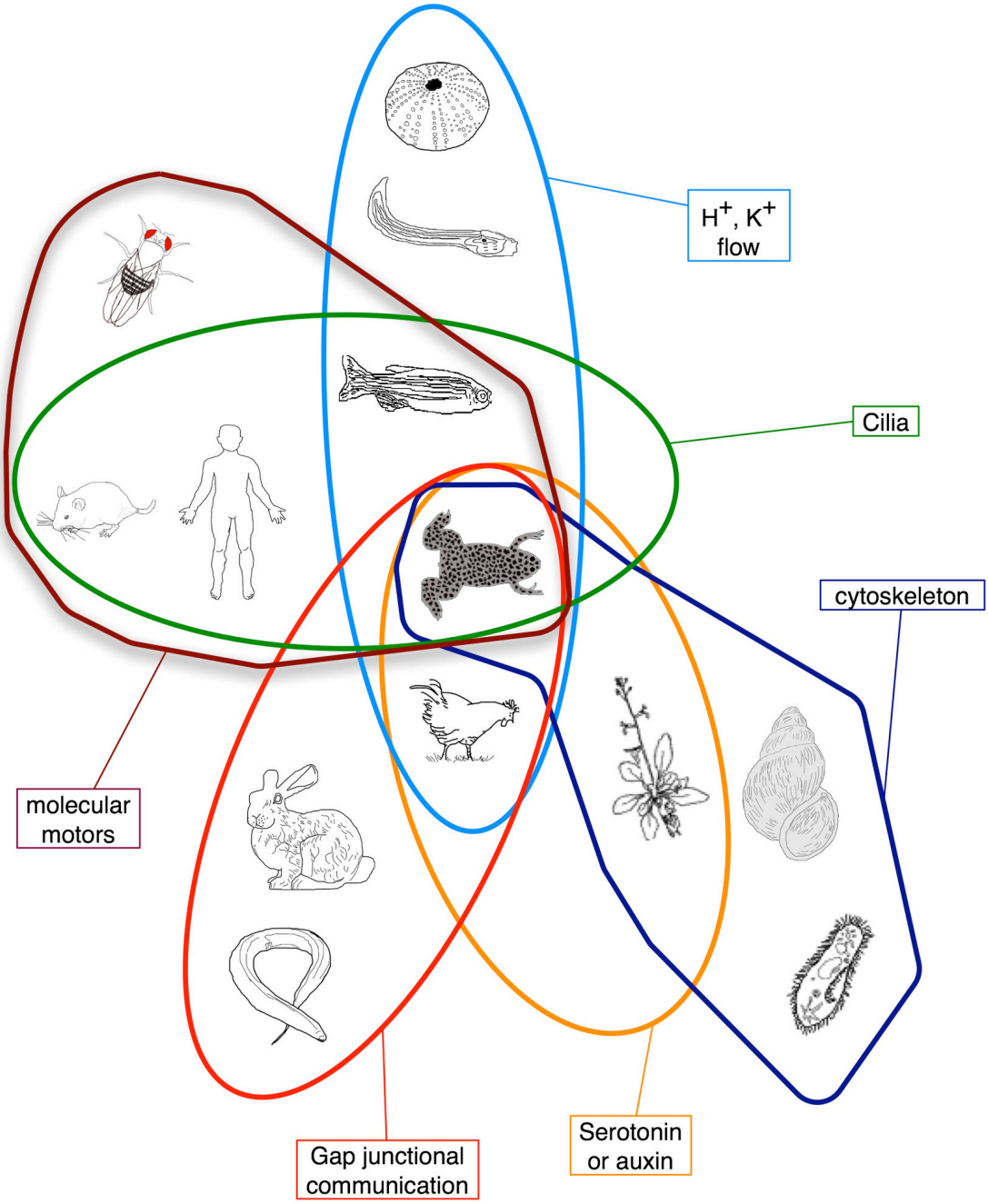

Figure 3.

Conserved mechanisms of LR patterning among phyla

Numerous mechanisms have been discovered that pattern the left-right axis in both vertebrates and invertebrates (Levin 2006): each of the organisms has at least one molecular component in common with one or more of the others (schematized by colored ovals encompassing the species that are known to utilize each mechanism).