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Left–right asymmetry: class I myosins show the direction

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Myosins are actin-based molecular motors that are found in almost all eukaryotes. Phylogenetic analysis allows the discrimination of 37 different types of myosins, most with unknown functions. Recent work in *Drosophila* has revealed a crucial role for type ID unconventional myosin in left–right asymmetry. Mutations in Myosin ID completely reverse the left–right axis (situs inversus), a phenotype that is dependent on an intact actin cytoskeleton. How this myosin might orient the left–right axis has began to be elucidated by showing that it interacts directly with β-catenin, suggesting that myosin ID interacts with the adherens junction to control the direction of organ looping. This is the first demonstration of a role of a myosin in body patterning.

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Introduction

In Bilateria, the establishment of a left–right (L/R) axis is essential for the organization and function of the brain and viscera [1,2]. In contrast to the symmetrical shape of the external body, the internal organs show complex patterns of L/R asymmetry in their morphology and position. Asymmetry arises during development following three basic morphogenetic processes: first, unilateral positioning — organs sit on one side relative to the midline, e.g. heart or spleen; second, directional looping — tubular organs (e.g. gut) coil in a stereotyped direction (dextral or sinistral); and third, handed differentiation, whereby bilateral organs, such as the brain and lungs, differentiate distinct left and right parts.

Symmetry breaking is the primary and essential step during L/R development. It takes place during embryogenesis, after the two main axes (anterior–posterior and dorsal–ventral) have been established [3••]. *De novo* asymmetry is then communicated to the whole body through identified cascades of asymmetric expression of genes, including the conserved nodal pathway [3••,4•,5]. How L/R asymmetry arises and how it becomes oriented relative to the two main axes are central issues. The fundamental problem of symmetry breaking was conceptualized early by Brown and Wolpert [6], who proposed that it could result from molecular chirality: they hypothesized that a handed molecule capable of self-orientation along the AP and DV axes could determine the L/R axis. This hypothetical molecule, termed the 'F-molecule' (each branch of a three-dimensional F defining one axis; see Figure 1), would be an L/R determinant, in other words a molecule that can decide what is left and what is right. Among other possibilities, it has been proposed that molecular motors capable of moving along polarized fibers could act as F-like molecules by moving unidirectionally toward one side of the cell [7].

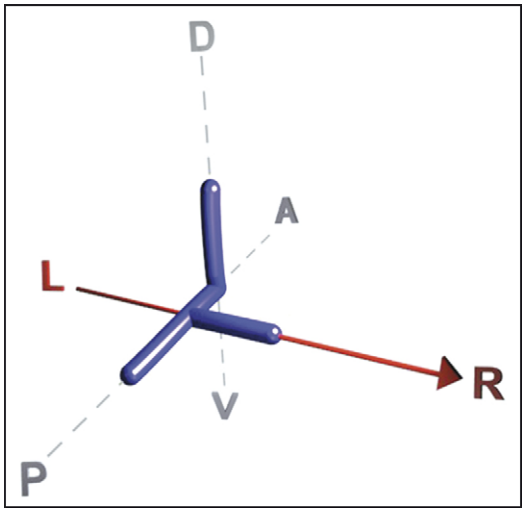
Various and elegant studies, mainly on vertebrate models, have proposed macromolecular structures as symmetry-breaking complexes [3••,4•]. In the mouse in particular, recent papers have established that rotating cilia present in the embryonic node generate a unidirectional fluid flow, which can move and concentrate molecules and/or vesicles asymmetrically in the node [8••–10••,11]. The fluid flow model is well supported by the fact that mutations in kinesin or dynein motors [12], which affect cilia formation or movement, respectively, can lead to L/R defects, as can experimental reversal of the nodal flow [13]. The finding that cilia are tilted posteriorly, coupled to their intrinsic chirality, makes them good candidates for F-macromolecular structures determining the L/R axis. Although cilia represent the earliest L/R patterning event in mouse, symmetry-breaking was shown to take place earlier than ciliary activity in *Xenopus*, fish and chick [3••,4•], suggesting the existence of distinct mechanisms to establish L/R asymmetry in vertebrates. More generally, whether vertebrates and other eukaryotes, including invertebrates, share common L/R-determining mechanisms is an important question.

Recently, a type I Myosin has been identified as being sufficient to orient the L/R axis in *Drosophila* [14••,15••]. This review will discuss this new role of myosin in determining body handedness.

The Myosin I family is involved in L/R determination in *Drosophila*

Unlike vertebrates, *Drosophila* does not have organs with prominent unilateral positioning. For example, the heart is a linear tube lining the dorsal midline. However, like vertebrates, wild type flies have tubular organs showing

Figure 1



Schematic representation of the 'F-molecule' as originally proposed by Brown and Wolpert [6]. Each branch of the molecule aligns with one of the three body axes. Following alignment with A/P and D/V axes, the F-molecule can orient the L/R axis because of its chirality. The directional activity of molecular motors make them potential F-molecules, provided they can orient according to A/P and D/V axes.

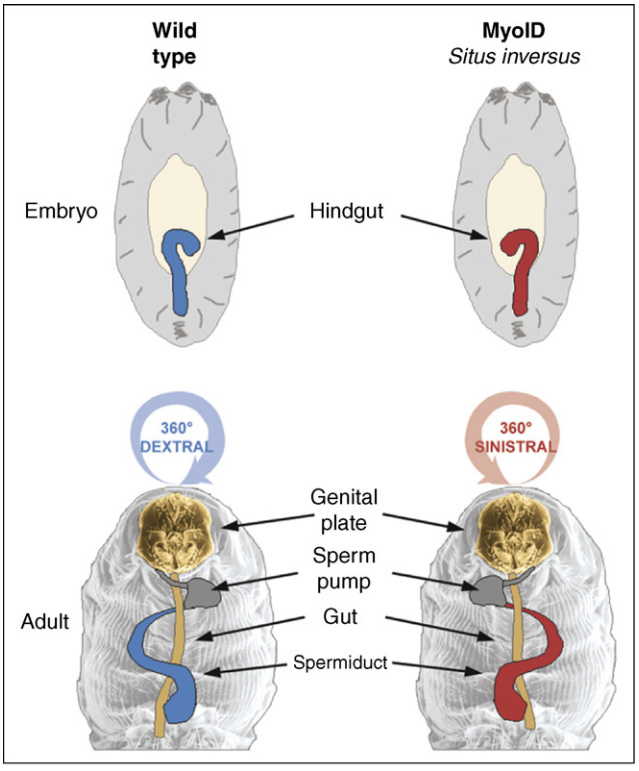
invariant coiling, including the gut and the testis [15••]. Additionally, the posterior-most part of the adult abdomen, the genital plate, undergoes a precise 360° clockwise (or dextral) rotation leading to the coiling of the spermiduct around the gut (Figure 2) [14••].

Genetic screening using the gut and spermiduct as phenotypic markers allowed two independent studies to identify Myo31DF as a situs inversus gene: the loss of Myo31DF function leads to a complete reversal of the L/R axis, with the gut, testis and spermiduct looping sinistrally rather than dextrally (Figure 2) [14••,15••]. These data demonstrate the existence of a genetically controlled L/R axis in *Drosophila*. They also provide the first evidence that an actin-based molecular motor plays a central role in orienting the L/R axis.

Using phylogenetic analysis of the motor domain, myosins have been classified into 24 main classes, some of them being found in very narrow species group (I to XXIV) [16••]. Further classification using associated protein domains allows the distinction of 37 different myosin types, whose evolutionary history has been determined [17••].

Drosophila Myo31DF belongs to the Class I / Type 12 myosin family [17••]. This class is characterized by a basic tail (TH1 domain) providing membrane-binding activity. Interestingly, Class I myosins are one of the few myosin classes (including TH1, MYTH4/FERM and SMC-DIL domain-containing myosins) that were recently proposed

Figure 2

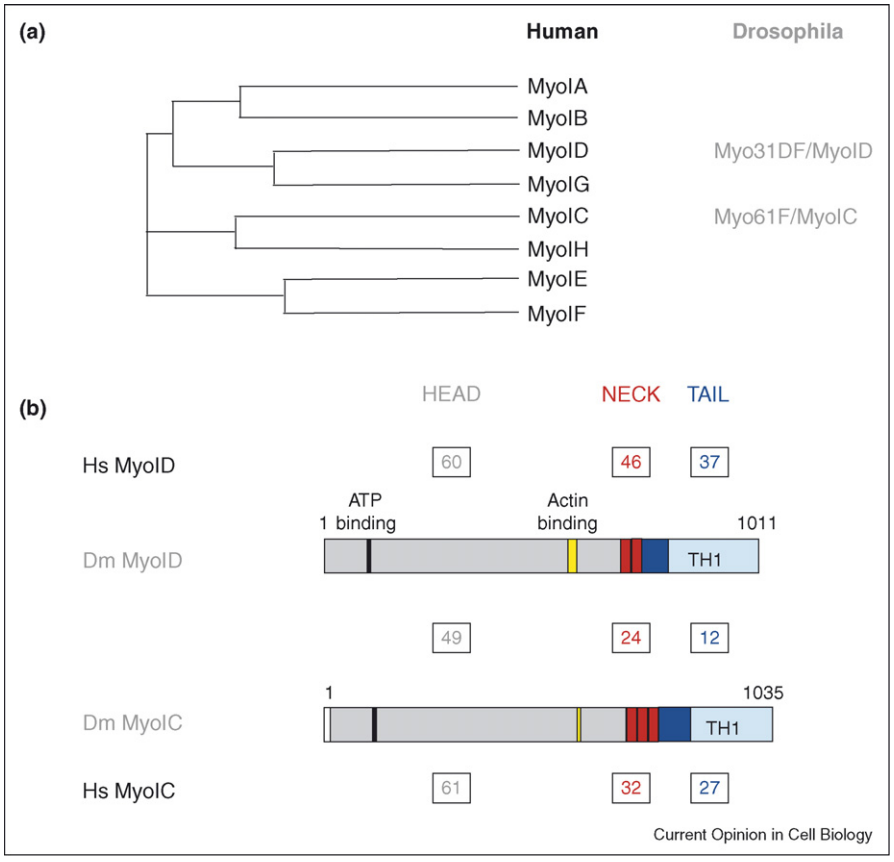


L/R markers in *Drosophila* and situs inversus phenotypes. L/R asymmetries in *Drosophila* result mainly from directional coiling of tubular organs, including the hindgut 'hook' in embryos (upper panel, dorsal view) and the spermiduct loop in adults (lower panel, ventral view, posterior up). Both phenotypic markers have a stereotyped, dextral orientation (blue) in wild-type flies, which is reversed to sinistral (red) in Myo31DF mutants. The looping of the spermiduct results from the external rotation of the genital plate, to which it is attached.

to be present in the last common eukaryotic ancestor (cenancestor) [17••], making Myosin I a possible ancestral L/R determinant during eukaryote evolution.

Class I myosins can be subdivided into long-tailed and short-tailed myosins. Long-tailed, ameboid-like myosins I are found essentially in protist and fungi and have been shown to control chemotaxis, pseudopod formation, actin nucleation and membrane trafficking. In this review, we will focus on short-tailed myosins in metazoa, which can be further divided into eight subclasses (A–H) [18]. *Drosophila* has orthologs only for type IC and type ID myosins, encoded by the Myo61F and Myo31DF genes, respectively (Figure 3) [19,20]. Type ID myosins are found in various species, from *C. elegans* to human, but their function is poorly characterized and only Myr4, the rat ortholog, was shown to be implicated in vesicular recycling [21]. Like other members of the myosin superfamily, Myo31DF is made of three structural domains: a head or motor domain, responsible for the generation of movement through actin-binding and ATPase activity; a

Figure 3



Myosins I in *Drosophila*. **(a)** The Human Myosin I class can be divided into eight subclasses (A to H) based on sequence phylogeny. *Drosophila* has orthologs for only Myosin IC and Myosin ID, encoded by the *myo61F* and *myo31DF* genes, respectively. The cladogram was calculated using the ClustalW program (default parameters). **(b)** *Drosophila* MyoID and MyoIC share a common, three-domain organisation: a head bearing the ATP- and the actin-binding sites (grey), a neck (red) with IQ domains and a tail containing a basic TH1 domain (blue). The neck domain contains a variable number of IQ motifs, two for MyoID versus three for MyoIC. The framed numbers indicate the scores calculated by the similarity matrix of the ClustalW program using default parameters (matrix Gonnet 250): the head domain is highly conserved between homologues, whereas the tail domain is more divergent.

neck domain, made of two IQ motifs that are likely to bind regulatory light chains; and a short tail domain (TH1) that is rich in basic amino acids (Figure 3). The tail domain, which is highly variable among myosins, is thought to provide functional specificity through selected interactions with other partners (cargoes).

Spatial and temporal requirement for Myo ID function

Genetic analysis of Myo31DF function reveals several interesting features. First, null mutations in Myo31DF are viable and fertile, with the only observed phenotypes being the reversal of L/R asymmetric organs. This finding suggests that Myo31DF has evolved into a specific L/R myosin that serves to orient organs properly. Whether such specialization is conserved among other organisms is currently unknown. Second, Myo31DF function is required within restricted regions of organ primordia and during a short period of time. The regions of Myo31DF activity are termed L/R organizers because they can direct the L/R development of the whole organ they control. A first organizer has been mapped to the hindgut and found to determine embryonic gut handedness [15••]; a second organizer was identified in the A8 segment of the larval genital disc, the precursor of the adult genitalia and spermiduct [14••]. Mosaic analysis in A8 showed that Myo31DF function has different roles in the anterior (A8a) and posterior (A8p) compartments. Myo31DF is essential in A8p to direct dextral development, while repressing sinistral development in A8a. This dual function is highly restricted temporally, as shown by temperature-shift experiments using a thermosensitive Myo31DF function; indeed, as little as three hours of Myo31DF function (compared to the 11 days of *Drosophila* development) are sufficient to orient the L/R axis properly [14••].

Importantly, Myo31DF is expressed symmetrically in the genitalia L/R organizer and its overexpression in the whole A8 segment does not affect L/R asymmetry. This feature, together with the spatial and temporal restriction of Myo31DF function, is expected of a determinant that must have the intrinsic ability to initiate asymmetric development from a symmetric state.

Actin in left–right asymmetry

The finding of Myo31DF as a L/R determinant suggests that the actin cytoskeleton plays a role in the process. The importance of actin during *Drosophila* L/R development has been investigated through indirect disorganisation of the actin network and genetic interactions.

Tissue-specific expression of dominant-negative forms of either small GTPases (Rho, Rac, Cdc42) or members of the JNK pathway, both regulators of the actin cytoskeleton, led to L/R defects both in the embryonic hindgut and during genitalia circumrotation [14••,15••]. Removing one copy of Myo31DF strongly enhanced the phenotype of either Drac1N17 or DJNKDN expression in the genitalia organizer. Furthermore, overexpression of moesin-GFP, an actin-binding protein, resulted in randomization of hindgut handedness. Interestingly, the timeframe requirements for Myo31DF function and moesin-GFP overexpression overlap, indicating a synchronous requirement for both Myo31DF and an intact actin cytoskeleton. Altogether, these data strongly suggest that normal actin organisation is essential for the development of L/R asymmetry in *Drosophila*.

The requirement for an actin network to allow L/R development is reminiscent of what is observed in another invertebrate, the freshwater snail *Lymnea*. The direction of shell coiling is highly stereotyped with most species showing dextral coiling [22]. Recent work showed that *Lymnea* snails require a proper actin cytoskeleton to undergo normal spiral cleavage in early embryos, which prefigures the direction of shell coiling [23]. Using latrunculin A or B to depolymerize actin, the authors have shown that a proper actin network is required for the correct dextral, helical positioning of micromeres at the eight-cell stage. Interestingly, depolymerization of microtubules using nocodazole treatment did not affect chirality of embryos, suggesting that the actin cytoskeleton, but not microtubules, plays a specific role in L/R asymmetry in snails.

In conclusion, the actin cytoskeleton appears to play a central role in L/R development in invertebrates, while in vertebrates, the microtubules (the major constituent of nodal cilia) seem to represent the main cytoskeleton network onto which asymmetry is set up. Whether the use of different cytoskeletons (actin versus microtubules) and their associated motors (myosins and kinesins) would represent a dichotomy between protostomes and deuterostomes to establish L/R asymmetry remains to be further investigated. In relation to this question, it is interesting to note that myosin and kinesin motors share a common origin [24,25].

Other Myo ID partners in L/R asymmetry: adherens junction, trafficking and calcium

One way to assess the functional role of myosins is to identify the direct partners, including cargoes, to which they bind through their tail domain. Previously, no Myo ID cargo had been identified, but recently a two-hybrid approach has been used to isolate several potential Myo31DF partners, including Armadillo (Arm), the *Drosophila* homolog of β-catenin, which links the actin cytoskeleton to the adherens junction [26]. Arm was shown using a GST pull-down assay to interact directly with Myo31DF *in vitro*. In addition, Myo31DF and Arm colocalize to the adherens junction in the genitalia L/R organizer, strongly suggesting that both proteins interact *in vivo* to orient the L/R axis [14••]. This apico-basal interaction could provide a way to orient Myo31DF perpendicular to the AP axis, along which Myo31DF function is polarized by way of compartmental-specific activity. A model has been proposed in which transient and oriented transport of cargoes to the adherens junction bias cell junctions to promote directional cell intercalation and/or sliding of genitalia cells, thus initiating dextral rotation of genital plate precursor tissues [14••]. Another myosin (myosin II) has already been shown to be able to control the pattern of junctions and to drive cell intercalation during embryonic development [27]. Interestingly, asymmetric development of the gut is accompanied by cell intercalation [15••], but whether cell intercalation is reversed in Myo31DF mutants and whether gut looping requires Arm remains to be demonstrated.

The interaction between Myo31DF and Arm is reminiscent of the interaction found between vertebrate inversin and β-catenin [28]. Inversin, an ankyrin-repeat containing protein whose function remains poorly understood, is the only known situs inversus gene in vertebrates, and thus could be considered as equivalent to Myo31DF [29,30]. (We are distinguishing genes whose mutation leads to 100% situs inversus from those leading to situs ambigus [randomization], i.e. mutations that can lead to a mix of situs solitus, situs inversus, heterotaxia and isomerism.) It is therefore tempting to suggest that both vertebrate and invertebrate situs inversus genes initiate L/R development through the adherens junction.

Another feature shared by Inversin and Myo31DF is the presence of two IQ domains [31]. The Myo ID IQ motifs are essential, since a neck-truncated form is not able to rescue the mutant phenotype [14••]. IQ domains can associate with regulatory light chains including calmodulin, making IQ-containing proteins potentially sensitive to calcium. Inversin has been shown to bind

calmodulin [31–33], and recent studies have implicated calcium signalling during early vertebrate L/R development [34,35]. It will be important to determine if Myo31DF responds to calcium to establish L/R asymmetry in flies.

Recent work in the mouse found a link between vesicles and L/R patterning. It showed that Hedgehog and retinoic-acid-containing vesicles called nodal vesicle parcels (NVPs) are present in the node and transported in the flow generated by cilia [10••]. Several myosins have been shown to transport vesicles [36], and, as mentioned previously, the MyoID rat homolog Myr4 participates in the recycling of the human Tranferrin receptor in MDCK cells [21]. Interestingly, expression of a dominant negative form of the *Drosophila* dynamin homolog, Shibire, leads to genitalia rotation defects, which are aggravated when one copy of the Myo31DF gene is removed [14••]. Thus, a possibility is that like in vertebrates, L/R factors are transported into vesicles to determine *Drosophila* asymmetry, potentially involving Myo31DF.

Two L/R myosins I?

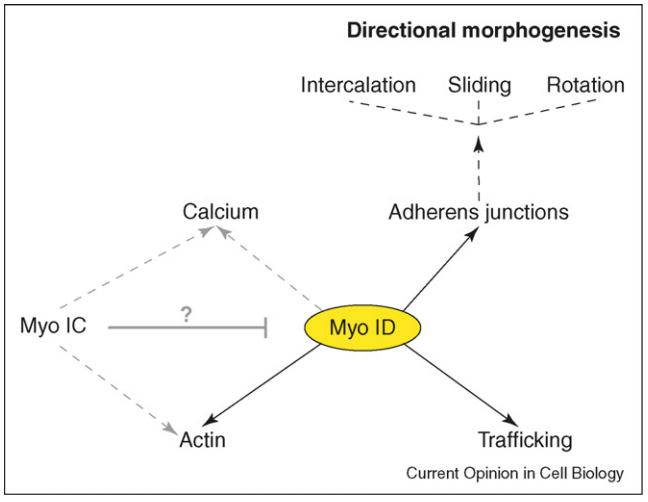
Data have clearly established a role for Myo31DF in L/R asymmetry, in particular in driving normal (i.e. dextral) development of looping organs. Interestingly, the study by Hozumi *et al.* [15••] provided some preliminary evidence showing that another closely related myosin, MyoIC/Myo61F, could play a role in L/R asymmetry. Experiments show that the overexpression of wild-type Myo61F in the embryonic hindgut and midgut leads to a full reversal of the handedness of both tissues, as is observed in MyoID mutants [15••]. This result suggests that Myo61F could have an antagonistic function over Myo31DF. Whether such an antagonism takes place normally during development remains to be established using Myo61F null mutants, which are not yet available. However, targeted expression of a Myo61F RNAi leads to few L/R defects, suggesting that Myo61F plays a role in gut asymmetry [15••].

MyoIC and MyoID proteins are very similar, displaying a highly conserved head and a short and basic tail (Figure 3). MyoIC has three IQ motifs in the neck domain, versus two for MyoID. One forthcoming challenge will be to understand not only the roles of MyoIC and MyoID, but also the molecular basis of the apparent antagonism between them to understand how two related class I myosins could control body patterning.

Conclusions

The study of reversed organ torsion in *Drosophila* mutants has allowed the discovery of a dextral determinant with actin-based motor activity (Figure 4). It emphasizes that actin is likely to play a central role in invertebrate L/R asymmetry, contrasting with the importance of the microtubule cytoskeleton in vertebrates. MyoID spatial organization along main axes and its intrinsic chirality towards actin filaments make it a good candidate for the F-molecule in *Drosophila*. Future studies will have to decipher the molecular and cellular mechanisms underlying L/R asymmetry in *Drosophila*, as well as to address their possible generalisation to other organisms.

Figure 4



Myosin ID is an essential L/R determinant in *Drosophila*. Molecular genetic data suggest a number of MyoID interactions with the adherens junction, trafficking, calcium and the other *Drosophila* Myosin I, Myo61F. See text for details.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Aylsworth AS: **Clinical aspects of defects in the determination of laterality**. *Am J Med Genet* 2001, **101**:345-355.

2. Bisgrove BW, Morelli SH, Yost HJ: **Genetics of human laterality disorders: insights from vertebrate model systems**. *Annu Rev Genomics Hum Genet* 2003, **4**:1-32.

3. •• Levin M: **Left–right asymmetry in embryonic development: a comprehensive review**. *Mech Dev* 2005, **122**:3-25.
This is a comprehensive review on left–right asymmetry, with in-depth discussion of the current data and models.

4. • Raya A, Belmonte JC: **Left–right asymmetry in the vertebrate embryo: from early information to higher-level integration**. *Nat Rev Genet* 2006, **7**:283-293.
This review discusses thoroughly the recent results and current models underlying vertebrate left–right asymmetry.

5. Raya A, Belmonte JC: **Sequential transfer of left–right information during vertebrate embryo development**. *Curr Opin Genet Dev* 2004, **14**:575-581.

6. Brown NA, Wolpert L: **The development of handedness in left/right asymmetry**. *Development* 1990, **109**:1-9.

7. Levin M: **Motor protein control of ion flux is an early step in embryonic left–right asymmetry**. *Bioessays* 2003, **25**:1002-1010.

8. •• Okada Y, Takeda S, Tanaka Y, Belmonte JC, Hirokawa N: **Mechanism of nodal flow: a conserved symmetry breaking event in left–right axis determination**. *Cell* 2005, **121**:633-644.
See annotation to [9••].

9. •• Nonaka S, Yoshiba S, Watanabe D, Ikeuchi S, Goto T, Marshall WF, Hamada H: **De novo formation of left–right asymmetry by posterior tilt of nodal cilia**. *PLoS Biol* 2005, **3**:e268.
This paper and [8••] address the mechanism by which the rotational movement of nodal cilia can generate a unidirectional flow. Direct observation of nodal cilia together with modelling allows the authors to show that cilia are tilted posteriorly in several vertebrate embryos, and that this inclining is the basis for unidirectional, rather than vortical, fluid flow. The orientation along the anterior–posterior axis together with their intrinsic chirality makes cilia good candidates as F-like structures.

10. •• 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**:172-177.
This paper describes the discovery of membrane-sheathed vesicles that are released in the nodal flow and liberate their content through fragmentation on the left side of the node. The particles are shown to contain the Sonic Hedgehog and retinoic acid molecules, and to require Fgf for their release. The data support a chemical, morphogen gradient mode of action of the nodal flow through the release of ligand-containing vesicles.

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

12. Brueckner M: **Cilia propel the embryo in the right direction**. *Am J Med Genet* 2001, **101**:339-344.

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

14. •• Speder P, Adam G, Noselli S: **Type ID unconventional myosin controls left–right asymmetry in *Drosophila***. *Nature* 2006, **440**:803-807.
This paper identifies Myo31DF/MyoID as a situs inversus gene in *Drosophila*. Myo31DF function is spatially and temporarily restricted to a L/R organizer in the precursor of the looping genitalia. β-catenin and Myo31DF are shown to physically interact *in vitro* and to co-localize *in vivo* at junctions, suggesting a conserved role for the adherens junction in establishing L/R asymmetry.

15. •• Hozumi S, Maeda R, Taniguchi K, Kanai M, Shirakabe S, Sasamura T, Speder P, Noselli S, Aigaki T, Murakami R *et al.*: **An unconventional myosin in *Drosophila* reverses the default handedness in visceral organs**. *Nature* 2006, **440**:798-802.
This paper identifies Myo31DF/MyoID as a L/R gene controlling gut and testis looping. Disruption of the actin cytoskeleton is shown to randomize gut looping. Overexpression of the related Myo61F/MyoIC myosin can antagonize Myo31DF/MyoID function, suggesting that the two *Drosophila* Myosin I genes function in L/R asymmetry.

16. •• Foth BJ, Goedecke MC, Soldati D: **New insights into myosin evolution and classification**. *Proc Natl Acad Sci U S A* 2006, **103**:3681-3686.
This paper presents a thorough phylogenetic analysis of the head-motor domain of known myosins, allowing the authors to propose an expansion of the current classification of myosins. Six novel myosin classes are described, and new protein domains associated with myosins are identified.

17. •• Richards TA, Cavalier-Smith T: **Myosin domain evolution and the primary divergence of eukaryotes**. *Nature* 2005, **436**:1113-1118.
This work uses myosin phylogeny to study early eukaryotic evolution and the unikont/bikont bifurcation. The analysis classifies myosins into 37 different types on the basis of combination of protein domains. In addition, it shows that the last common eukaryotic ancestor had a complement of myosins with three distinct domains, including myosin I.

18. Gillespie PG, Albanesi JP, Bahler M, Bement WM, Berg JS, Burgess DR, Burnside B, Cheney RE, Corey DP, Coudrier E *et al.*: **Myosin-I nomenclature**. *J Cell Biol* 2001, **155**:703-704.

19. Morgan NS, Skovronsky DM, Artavanis-Tsakonas S, Mooseker MS: **The molecular cloning and characterization of *Drosophila* melanogaster myosin-IA and myosin-IB**. *J Mol Biol* 1994, **239**:347-356.

20. Morgan NS, Heintzelman MB, Mooseker MS: **Characterization of myosin-IA and myosin-IB, two unconventional myosins associated with the *Drosophila* brush border cytoskeleton**. *Dev Biol* 1995, **172**:51-71.

21. Huber LA, Fialka I, Paiha K, Hunziker W, Sacks DB, Bahler M, Way M, Gagescu R, Gruenberg J: **Both calmodulin and the unconventional myosin Myr4 regulate membrane trafficking along the recycling pathway of MDCK cells**. *Traffic* 2000, **1**:494-503.

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

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

24. Kull FJ, Sablin EP, Lau R, Fletterick RJ, Vale RD: **Crystal structure of the kinesin motor domain reveals a structural similarity to myosin**. *Nature* 1996, **380**:550-555.

25. Kull FJ, Vale RD, Fletterick RJ: **The case for a common ancestor: kinesin and myosin motor proteins and G proteins**. *J Muscle Res Cell Motil* 1998, **19**:877-886.

26. Tepass U: **Adherens junctions: new insight into assembly, modulation and function**. *Bioessays* 2002, **24**:690-695.

27. Bertet C, Sulak L, Lecuit T: **Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation**. *Nature* 2004, **429**:667-671.

28. Nurnberger J, Bacallao RL, Phillips CL: **Inversin forms a complex with catenins and N-cadherin in polarized epithelial cells**. *Mol Biol Cell* 2002, **13**:3096-3106.

29. Morgan D, Turnpenny L, Goodship J, Dai W, Majumder K, Matthews L, Gardner A, Schuster G, Vien L, Harrison W *et al.*: **Inversin, a novel gene in the vertebrate left–right axis pathway, is partially deleted in the inv mouse**. *Nat Genet* 1998, **20**:149-156.

30. Eley L, Turnpenny L, Yates LM, Craighead AS, Morgan D, Whistler C, Goodship JA, Strachan T: **A perspective on inversin**. *Cell Biol Int* 2004, **28**:119-124.

31. Morgan D, Goodship J, Essner JJ, Vogan KJ, Turnpenny L, Yost HJ, Tabin CJ, Strachan T: **The left–right determinant inversin has highly conserved ankyrin repeat and IQ domains and interacts with calmodulin**. *Hum Genet* 2002, **110**:377-384.

32. Yasuhiko Y, Shiokawa K, Mochizuki T, Asashima M, Yokoyama T: **Isolation and characterization of Xenopus laevis homologs of the mouse inv gene and functional analysis of the conserved calmodulin binding sites**. *Cell Res* 2006, **16**:337-346.

33. Yasuhiko Y, Imai F, Ookubo K, Takakuwa Y, Shiokawa K, Yokoyama T: **Calmodulin binds to inv protein: implication for the regulation of inv function**. *Dev Growth Differ* 2001, **43**:671-681.

34. Raya A, Kawakami Y, Rodriguez-Esteban C, Ibanes M, Rasskin-Gutman D, Rodriguez-Leon J, Buscher D, Feijo JA, Izpisua Belmonte JC: **Notch activity acts as a sensor for extracellular calcium during vertebrate left–right determination**. *Nature* 2004, **427**:121-128.

35. Shimeld SM: **Calcium turns sinister in left–right asymmetry**. *Trends Genet* 2004, **20**:277-280.

36. Seabra MC, Coudrier E: **Rab GTPases and myosin motors in organelle motility**. *Traffic* 2004, **5**:393-399.