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Centriole enantiomerism: unexpected information from mice and fish

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Abstract

In mice, asymmetry of internal organs is established by a left-ward fluid flow, produced by the innermost monociliated cells of the node and sensed by the immotile primary cilia of left peri-nodal cells; thus, the Nodal signaling pathway is asymmetrically (left-sided) expressed. However, right peri-nodal cells also, if excited by an artificial right-ward flow, break symmetry and activate the Nodal cascade, though inverting the asymmetry of visceral organs (*situs inversus*): peri-nodal cells prove to be adept at distinguishing flow directionality; moreover, the same signaling pathway is identically triggered in left and right peri-nodal cells, producing two symmetric architectural results: one program, two different implementations, one input, two mirror outputs. Recently, in the Kupffer's vesicle (the laterality organ of zebrafish), chiral cilia orientation has been described: primary cilia, in left and right half, are mirror oriented relative to the midline. Do Kupffer's vesicle histology and perinodal cell mirror behavior suggest primary cilia are enantiomeric organelles?

Key words

Centriole, centrosome, bilateral symmetry, symmetry breaking, primitive node.

Symmetry and symmetring breaking

Centrosomes (cells have only one centrosome as only one nucleus, except during mitosis) are metazoan organelles, roughly spherical, made up of a pair of centrioles, orthogonal during S and M phase and, sometimes, in interphase, embedded and immersed in an orderly structured matrix of proteins, called PeriCentriolar Material (PCM): from its surface several molecular platforms, named γ-Tubulin Ring Complexes (γ-TuRCs), nucleate an aster of MicroTubules (MTs) spherically radiating from the centrosome PCM lattice toward the cell cortex; the aster comprises MTs nucleated directly by centrosome γ-TuRCs or starting from the wall of centrosomal MTs (Sánchez-Huertas and Lüders, 2015), or from augmin-dependent Microtubule Organizing Centers (MTOCs). Centrosomes comprise two centrioles, orthogonally arranged in the form of an uppercase letter 'L' during the S, G2 and M phase: one centriole, axial, is named 'Mother' (MC) and is equipped with two sets of 9 appendages, distal and subdistal, while the second centriole, at the proximal end of the MC, called 'Daughter' (DC), shows 9 distal outgrowths or ribs, but is appendage free (Vorobjev and Chentsov, 1982). DC "migrates extensively throughout the cytoplasm" (Piel et al., 2000), yet actively cooperates in S phase to form the structure of PMC and in G1 to assemble the primary cilium (Loukil et al., 2017).

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Primary cilia. Motile cilia and flagella show 9+2 geometry (9 peripheral MT doublets, plus two inner MTs); differently, primary cilia are non-motile, except, almost, in the innermost part of the Node (9+0 symmetry, no inner MTs): cells have only one cilium, as only one centrosome, formed by the MC embedded in the centrosomal PCM that remains attached at its base; cilium and PCM are resorbed and degraded before mitosis; primary cilia act as external antennae (chemical signaling pathways and mechanosensitive functions). Besides MC, also the DC plays a fundamental role in the process of cilium assembly (Loukil et al., 2017).

Centrosome duplication cycle: the two centrioles, MC and DC, enter the S phase completely disengaged, the 'Daughter' matures, acquiring distal appendages, thus becoming itself a 'Mother'; then, from each Mother a new DC arises, orthogonally, using its Mother as a platform, not as a template; PCM proteins are then recruited, orderly assembled and two new centrosomes are generated at cell division, one per cell.

The mouse unpair Node, formed on the midline, at the posterior end of the notochord (Lee and Anderson, 2008) is composed of innermost cells (pit cells, so called because of the shape of the piriform concave mouse node) characterized by a long primary (9+0) rotating cilium on their apical surfaces. The node central pit is surrounded by 20–30 peripheral monociliated, non-motile but passively flexible cells, called crown cells and preferentially localized on the left and right borders of the node.

Bilateral symmetry, in terms of evolution, is a very successful trait, shared by almost all Metazoa (Bilateria), very ancient (Urbilateria), ideal for the balance and mechanical stability of locomotive systems (walking, running, swimming, flying, sidewinding) and for the differential analysis of the perceived stimuli by sensorineural apparatuses (detection of visual and acoustic stimuli source location).

Symmetry breaking. Mirror symmetry, however, is not a good choice for internal organs not in charge of motion or perception: in fact it is early broken to solve architectural anatomical problems (dramatic in narrow snake bodies) and functional challenges (hydrodynamics of double circulation, gut length); so, during development, bilateral symmetry is, in a first step, early imposed and later, very soon cancelled in visceral organs "to maintain organ asymmetry in otherwise perfectly bilaterally symmetric Vertebrates" (Blum et al., 2014). Symmetry-breaking process is quite complicated in some Echinoderms (starfish): bilaterally symmetric *larvae* acquire an apparent radial symmetry through an enormously amplified Left-Right asymmetry strategy (Morris, 2007): they seem to have adopted an unusual circular pentamerism, different than the straight metamerism of Insect segments and Vertebrate somites.

Bilaterian bodies, seen from the outside, are then mirror symmetric, but visceral organs are mostly unpaired and not bilaterally symmetric: in Vertebrates, usually (*situs viscerum solitus*) heart and spleen are located in the left side, but liver, stomach and pancreas are right-sided; gut and lungs are not symmetric and not sagittal. However, the reverse disposition of only some internal organs, known as *situs viscerum inversus*, represents a severe pathology, whereas the complete inversion of *viscera* (*situs viscerum totalis*) does not create any abnormality.

How is asymmetry of visceral organs established? Tanaka et al. (2005) suggested that a ciliary flow produced by node pit cells may create a morphogen gradient. Also an electrophoretic movement of an asymmetry determinant has been hypothesized.

In chick (whose Hensen's node cells lack motile cilia as in pig) and in frog the neurotransmitter serotonin seems to have some role in symmetry breaking upstream of asymmetric gene expression and before the appearance of cilia. However, morphogens or determinants have never been identified, the details of serotonin's movement in chick and other species are unclear.

How is asymmetry of visceral organs established in mice? In mouse embryos, during gastrulation, the three primordial germ layers originate through an epithelial to mesenchymal transition and invagination of epiblast cells that occurs at the primitive streak on the midline: the node, a little group of cells positioned at the anterior end of the primitive streak, determines left right asymmetry of internal organs. Node cells possess on their apical side one primary cilium: primary cilia of the innermost cells (pit cells) are motile whereas peripheral cells (called crown cells) have immotile cilia; pit cells produces a fluid flow sensed by crown cells: two hypotheses about this mechanism have been proposed: perinodal cells can be bent and mechanically stretched (opening cation channels) or can sense molecular gradients (although never identified).

In the mouse node, motile primary cilia of inner cells rotate clockwise (viewed from above their apical side), as in zebrafish Kupffer's vesicle (Okabe et al., 2008), thus producing a fluid flow directed to the left side of the node: here monociliated peri-nodal 'crown' cells likely work as mechanosensitive devices (Praetorius and Spring, 2001); Polycystin-2 (PKD2) cation channels, localized on their cilia, are stretch-activated when cilia are bent by the nodal flow (Yoshiba and Hamada 2014) thus inducing Nodal expression in their cytoplasm and then activating Nodal pathway expression only in the left lateral plate mesoderm, and realizing the usual asymmetry of visceral organs (*situs solitus*). To ascertain the role of the nodal flow, Nonaka and co-workers (2002) and Yoshiba et al. (2012) cultured in wide flow-chambers several mouse embryos, fixed in parallel rows, coordinately and correctly oriented so that crown cells of each node were exposed together, and simultaneously subjected, to artificial laminar flows, alternately left- and right-ward. Right peri-nodal cells, which also have ciliary-positioned PKD2 cation channels (Yoshiba et al., 2012), responded to artificially induced right-ward flows, inducing Nodal expression in their cytoplasm and then activating Nodal pathway expression only in the right lateral plate mesoderm just like left cells responded to left-ward flows: perinodal cells can distinguish flow directionality, showing mirror differential sensibility to oppositely directed flows: left crown cells are excited by left-ward flows, right cells by right-ward. Moreover, both left and right peri-nodal cells, excited by proper flows, trigger asymmetric expression of the Nodal cascade, but inversely: Right crown cells activate the Nodal pathway in the right lateral plate mesoderm, producing *situs inversus*, the mirror image of *situs solitus*: internal organs are mirror shaped and reversed from their usual positions: one genomic pathway, two bilaterally symmetric realizations. It is worth mentioning that these experiments challenge the morphogen hypothesis: node-secreted signaling substances, flow transported, would invest left and right cilia of all nodes with increasing gradient; the hypothesized chemosensors, anyway, should at least be mirror positioned on left and right crown cell cilia.

In the zebrafish laterality organ, Kupffer's vesicle, Ferreira et al. (2018) measured motile and immotile cilia orientation relative to the midline discovering that in the left and right side of the early (3 somites stage) vesicle primary cilia orientation is

markedly mirror symmetric: motile cilia: +14° on the left side, -21° on the right; later (8-14 somites stage) cilia orientation rotates 20° toward the right, showing a dextral orientation over the whole vesicle, yet maintaining the same angular difference between the left and right side. Cilia of laterality organs in mice and fish appear morphologically and physiologically mirror symmetric.

Mechanism(s) of symmetry breaking

In this paper I'll not consider the process of symmetry breaking *'per se'* but how the Nodal cascade is triggered in only one side and how primary cilia can realize this process.

In experimental flow chambers, each artificial fluid flow, left- or right-ward, bends crown cells cilia of the same node oppositely relative to the embryo (and node) midline: a left-ward flow bends primary cilia of left-sided peri-nodal cells away from the midline and these cells respond by opening PKD2 ion channels (then *situs solitus*); the same left-ward flow bends (oppositely) primary cilia of right-sided peri-nodal cells toward the midline, and these cells do not respond; the opposite right-ward flow bends toward the midline primary cilia of left peri-nodal cells, that are not excited, while right crown cells, whose cilia are bent away from the midline by this right-ward flow, respond by opening PKD2 ion channels (then *situs inversus*): peri-nodal cells respond only to self-ward flows coming from the midline: primary cilia of peri-nodal cells (left- or right-sided) may be bent both toward the mid line and away from it, but only if they are bent away from the midline then peri-nodal cells open their PKD2 cation channels. Strikingly peri-nodal cells prove to be adept at distinguishing flow directionality.

The mouse node is an array made up of 150-250 antero-posterior polarized cells (Hashimoto and Hamada, 2010): basal bodies of motile primary cilia must be absolutely posterior-positioned for a laminar flow to be produced by an ordered array of monociliated cells (Nonaka et al., 2005; Hirokawa et al., 2006): planar cell polarity (PCP) establishes antero-posterior polarity of nodal cells and provides the posterior positioning and tilt to node cilia (Hashimoto et al. 2010); as known, PCP (non-canonical Wnt pathway) is necessary for the common and shared orientation of cells within the plane of an epithelium. In the mouse node, Cofilin 1, an actin-severing protein, together with Vangl2, a core PCP protein, cooperate to control posterior positioning of cilia on pit-cells, essential for the initiation of left-right asymmetry (Mahaffey et al. 2013). Node cilia appear correctly positioned in Cofilin 1 and Vangl2 single mutants, while are center-positioned in double mutants (*situs inversus*). PCP polarizes antero-posteriorly also the cells of *Xenopus laevis* gastrocoel roof plate, the laterality organ of frogs (Antic et al., 2010) as the dorsal forerunner cells, precursors of Kupffer's vesicle in zebrafish *Danio Rerio* (Oteiza et al., 2010).

As seen, peri-nodal cells, antero-posteriorly polarized and aligned by PCP, are excited only when their cilia are bent away from the embryo midline by self-ward directed flows: the rationale behind their mirror response is that primary cilia of left and right crown cells are each other mirror symmetric, so that their PKD2 cation channels, mirror positioned relative to the embryo midline, are excited by oppositely directed flows. This thesis is supported by the cited recent finding of Ferreira et al. (2018) in the zebrafish Kupffer's vesicle.

Primary cilia are assembled by the mother centriole of an integer centrosome: cells containing only the mother centriole cannot form the primary cilium (Loukil et al., 2017); a role of centrioles and centrosomes in Left-Right patterning is not a new topic: Vanderberg and Levin (2009) suggested that "the coordination of the 3 axes is performed by a cytoskeletal organizing center such as the centriole or basal-body: a sharp midline separation is already evident after the first cell cleavage in Xenopus and Left and Right blastomeres inherit immediately differential chiral information, then transmitted to the progeny" (see also McDowell et al., 2016); Xu and colleagues (2007) proposed an autonomous "intrinsically chiral structure, perhaps the centrosome, serving as a template for managing polarity in the absence of spatial cues: such a template could help to determine Left– Right asymmetry and mirror planar polarity in development". Mouse node and zebrafish Kupffer's vesicle show how centrioles and centrosomes may work in Left-Right patterning: peri-nodal cells are capable of distinguishing flow directionality because of mirror symmetry of their primary cilia (and then of centrioles and centrosomes).

Geimer and Melkonian (2004; 2005), reviewed by Marshall (2012), Pearson, (2014) and Dutcher and O'Toole (2016), described inside the basal-body of Chlamydomonas "an 'acorn-like' asymmetric structure, adhering in a highly inter-individual reproducible and invariable manner to triplets N. 2-1-9-8-7" and another structure, shaped like the uppercase letter 'V' (centrin V-fiber) in contact with triplets N. 9, 5 and 4: "Whereas the cartwheel is thought to nucleate the nine-fold rotational symmetry of the microtubular triplets, the acorn might play an equally important role imposing rotational asymmetry on the microtubular triplets, perhaps leading to the asymmetric assembly of basal-body-associated fibers and hence cellular asymmetry in general" (Geimer and Melkonian, 2004). A linear marker of "rotational asymmetry" (recently, Tovey and coworkers, 2018, have ascertained γ-Turc heterogeneity in flies,) can be arranged clockwise or counter-clockwise like a belt, thus originating two enantiomeric rings, inversely polarized; it seems that the 9-fold architecture of ciliary locomotive machinery has been utilized to build discrete geometric tools, circumferentially polarized for carrying out finely tuned directional tasks: centriole 9-fold architecture has been adopted by distal and subdistal appendages of mother centrioles, transition fibers of basal bodies, Y-shaped linkers of the cilium transition zone, pericentriolar material of centrosomes, whose 9-scaffold toroidal geometry is realized by pericentrin (Mennella et al., 2012; Mennella 2014).

Bilateral symmetry is the main characterizing property of Bilateria: it is the best solution for empowering efficient and balanced locomotive systems able to rapidly drive movement direction and sensorial systems that guarantee good spatial orientation (Regolini, 2013); bilateral symmetry is imposed very early in Bilateria embryos and soon broken in some nascent organs to solve difficult problems (cardiovascular hydrodynamics, length of digestive apparatus): it is not surprising that imposition and breakage of bilateral symmetry is sustained by the same organelles.

In amphioxus, manipulating the Nodal cascade, Li et al. (2017) obtained '2- Left' or '2- Right' phenotypes: '2-Left' mutants duplicate the wild-type left-sided organs and lose the right-sided ones: instead of their typical mouth only on the left side, '2-Left' mutants have two, mirror symmetric, "paired bilateral mouths". Similarly, in human cardiac isomerism two right or two left atria develop, and in both cases, the two atria are mirror-images of each other (Hildreth et al., 2009).

Organ shape depends on the side from which organs or parts of them originate, where specific morphogenetic genes are switched on (the Nodal cascade is an example): when genes are switched on, both left- and right-sided cells accomplish their task, but mirror symmetrically: right-sided cells build only right-handed structures and left-sided cells build only left-handed structures. In Bilateria, paired or unpaired, internal or external organs, or parts of organs as in cardiovascular, respiratory and digestive apparatuses, developed from left-sided fore runner cells are precise mirror images of the same organs, or their parts, developed from right-sided fore runners: in *situs inversus totalis*, right-handed heart and great vessels, respiratory and digestive apparatuses are perfect mirror images of the same left-handed organs of *situs solitus*; axial structures (notochord, neural tube) are formed by fore runner cells hailing from the left and right side of the embryo, whose behavior is perfectly mirror symmetric. Fingerprints and human cortical gyri are exceptions not considered because non-genetic factors intervene.

Bilateria show two symmetric ways in which cells are assembled for building organs: left- and right-handedness (sinistrality or dextrality); to be capable of building left- or right-handed organs, the only rationale is that they must necessarily possess symmetric geometric tools (likely the centrioles) that drive the symmetrical location of cell-cell junctions and cell receptors for extracellular fiber matrix.

Intriguingly, Li et al. (2019) presented a robotic system controlled by exploiting statistical mechanics phenomena, by incorporating many loosely coupled 'particles', very similar to centrioles.

A question arises: do laterality organs literally 'break' bilateral symmetry, or do they simply establish in which half of the embryo some genes of fore runner cells (buds or primordia) of some visceral organs are allowed (or forbidden) to start, thus determining the left or right handedness of their future shape? Bilateral symmetry of *situs solitus*/*situs inversus* supports the second hypothesis; symmetry is not 'broken': establishing the side where fore runner cell genes are switched on, it is selected which of the two possible shapes (Left or Right) will be realized: The Nodal pathway can be expressed only in the left plate mesoderm (*situs solitus*), only in the right plate mesoderm (*situs inversus totalis*) or in both left and right plate mesoderm (*situs inversus* or *situs ambiguous* or heterotaxia); individuals with *situs inversus totalis* phenotype are perfectly healthy.

Bilateria organs can be divided in three groups: *i*) paired (limbs, eyes): sinistral and dextral bilaterally symmetric structures are expressed far apart; ii) axial (skull, chest, vertebral column, urogenital apparatus): sinistral and dextral bilaterally symmetric halves are built and fused together on the midline; iii) unpaired (heart, lungs, gut) that may be realized in two mirror configuration, *situs solitus*/*situs inversus*, depending on the side in which they are built.

In bilarerians we find the same Hox genes in right and left halves: *Nematostella vectensis* knockdown-types (short hairpin RNAs and CRISPR-Cas9) show anomalies in body and limbs patterning (number or size) but express the same Hox genes bilaterally. (He et al., 2018)

Albeit genes and DNA are identical in each cell of the same organism, the 'ideal, virtual' shape of every organ can be 'actually' realized as left-handed (sinistral) or right-handed (dextral) depending on the side from which precursor cells derive: mother centrioles seem chiral tools, bilaterally symmetric, responsible for driving (mirror symmetrically) growth and form of organs in Bilateria: centrioles may be the

chiral structures capable of mirror implementation and translation of the same gene instructions. Therefore, one possibility (as crazy as logical, but not counterintuitive) is that, in left and right halves of Bilateria, left ('levo', '+') and right ('dextro', '-') enantiomeric mother centrioles (and then basal bodies, primary cilia, centrosomes) do exist. After all, centrioles show the highest micro- macro-scale correlation: they organize pericentriolar material, asters and sister asters, and, in mitosis, by the peculiar centrosome duplication cycle, the cytoskeleton of daughter cells is patterned upon that of their mother (planar cell chirality).

Conclusion and perspectives

Mice peri-nodal cells prove to be adept at distinguishing flow directionality; in Kupffer's vesicle primary cilia in left and right half, are mirror oriented relative to the midline; mice perinodal cell mirror behavior together Kupffer's vesicle histology suggest primary cilia are enantiomeric geometric organelles. Bilateral symmetry and functioning of actual tissues and organs, like the mouse node and zebrafish Kupffer's vesicle, support this idea that centrioles and centrosomes are enantiomeric; moreover, an orchestrated interplay of PCP, primary cilia and centrosomes in tissue polarization, much more pronounced than so far thought and capable of reaching highly and finely tuned directional anisotropic (symmetric and antisymmetric) results, emerges. Mice and fish information is a strong challenge to our knowledge. Are stem cells chiral? Is their behavior intrinsically different, Left or Rigth?

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