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Left-right symmetry breaking: learning from the chicken

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Morphological left-right asymmetry of visceral organs in most cases reveals a typical arrangement. This implies directed symmetry breaking which is suggested to be based on the existence of structural chirality. At early developmental stages many vertebrate model organisms display so-called leftward flow of extracellular fluid which is based on the unidirectional rotation of chiral cilia. Cytoskeletal chirality has been shown to contribute to the left-right asymmetry of invertebrates including *Caenorhabditis elegans* and *Drosophila melanogaster*. The mechanisms of left-right symmetry breaking in vertebrates without ciliary flow remain mysterious. Here, we present our perspective on left-right patterning and symmetry breaking in the chick within a broader context.

KEYWORDS

left-right symmetry breaking, gastrulation, chirality, vertebrate embryo, cytoskeleton

Meeting point nodal

Left-right asymmetry of viscera is a fundamental anatomical feature of vertebrates and many invertebrates (Blum and Ott, 2018). Morphological asymmetry is manifested both in bilateral asymmetry or handedness of organs and their asymmetric position and was shown to be preceded by asymmetric gene expression and asymmetrical molecular signaling (referred to here as *left-right patterning*). Expression of TGF-beta member Nodal in the left lateral plate mesoderm is a common denominator of early left-right molecular patterning in studied vertebrates (Duboc and Lepage, 2008; Grande and Patel, 2009). Well-studied effector of asymmetric Nodal activity is the transcription factor Pitx2, which has been shown to be directly involved in asymmetric morphogenesis of the heart and the gut (Campione et al., 1999; Lin et al., 1999). Interestingly, the lateral plate mesoderm is involved in the morphogenesis of asymmetrical organs contributing to heart and connective tissues of the gut. Nodal-Pitx2 module was also reported to be involved in asymmetrical development of amphioxus, snails and sea urchins. Asymmetrical gene expression, however, is preceded by a symmetrical state, displaying either bilateral absence or bilateral presence of gene expression or molecular activity. Assuming original bilateral symmetry of a developing embryo, asymmetric gene expression is preceded by a process described as initial symmetry breaking which is a transition to a state with higher symmetry.

The (chiral) form is the cause

The original idea proposed by Pierre Curie suggested that the symmetry elements in the cause must be found in their effects and asymmetries in the effect are derived from asymmetries in the causes (Curie, 1894). Further development of the symmetry breaking concept led to distinction between explicit symmetry breaking, which follows the above described definition and spontaneous symmetry breaking (Earman, 2004) where

the outcomes are distributed equally (outcomes are randomized). An important feature of animal left-right symmetry breaking, however, is its directionality: the resulted sidedness after the symmetry breaking is not random. Hence, the initial animal left-right symmetry breaking cannot be described as spontaneous. This suggests underlying structural molecular asymmetry which may be based on chirality as known in chemistry. Wolpert and Brown (Brown and Wolpert, 1990) proposed the existence of a chiral molecular determinant denominated as F-molecule which generates initial asymmetry subsequently translated at cellular and organismal level. Afzelius reported a correlation between disturbed laterality and abnormal cilia (Afzelius, 1976): ultrastructural examination of immotile sperm in 4 patients with impaired mucociliary transport and associated recurrent bronchitis revealed the absence of dynein arms in the cilia with three out of four patients also showing situs inversus totalis. The association of bronchiectasis accompanied by sinusitis with situs inversus was already described by Manes Kartagener (Kartagener and Stucki, 1962). Afzelius proposed that the cilia motility in embryonic tissues critically contributes to the right-left asymmetries of adult organisms (Afzelius, 1976). As cilia reveal structural chirality they fulfill requirements of the hypothetical F-molecule. Indeed, shortly after the proposed role of the hypothetical F-molecule, motile cilia were shown to be present in early mouse embryos during gastrula and early somitogenesis: the ventral surface of epithelialized midline cells assigned to the notochord and node possess long motile monocilia (Sulik et al., 1994). Based on results of Afzelius and observation that the comparable developmental stage in rat embryos is critical for the establishment of right-left asymmetry (Fujinaga and Baden, 1991), Sulik and co-workers suggested a causal contribution of motile monocilia to left-right symmetry breaking (Sulik et al., 1994). Screening of mouse strains with complete inverted viscera revealed mutations in cilia-associated axonemal heavy-chain dynein (Supp et al., 1997). Similarly, it was shown that mice depleted for kinesin KIF3B do not form monocilia and reveal randomized left-right asymmetry (Nonaka et al., 1998). Furthermore, in WT embryos corresponding cilia undergo rotation accompanied by leftward fluid flow as shown by video microscopy of ventral surface of embryos cultured in a medium with fluorescent beads. The experimental reversal of flow direction led to the formation of situs inversus while an artificially generated flow in embryos with defective cilia “rescued” the phenotype (Nonaka et al., 2002).

The direction (of flow) decides where it becomes the left side

Leftward flow was observed in frog *Xenopus laevis*, zebrafish and mouse while morphological signs of a ciliated organizer were reported for many further but not for all studied vertebrates (Schroder et al., 2016; Blum et al., 2007). Successful symmetry breaking requires a temporary formation of a specific “organ”: the ciliary leftward flow is located at the ventral surface of specialized structure called left-right organizer (LRO, cf. Figure 1). Crucially, symmetry breaking by leftward flow is followed by subsequent activation of nodal signaling at the left-side (Schweickert et al., 2010; Oki et al., 2009). Two mechanisms were proposed to explain how leftward flow caused left-right patterning. Initial proposal suggested

leftward transport of morphogens (Nonaka et al., 1998) while later studies suggested existence of sensory immotile cilia (McGrath et al., 2003; Tabin and Vogan, 2003). Indeed, recent studies in mouse and zebrafish embryo strongly support the view that deflection of immotile cilia by flow which lead to polycystin-2 (Pkd2) channel mediated activation of intraciliary Calcium transients (Katoh et al., 2023; Djenoune et al., 2023). How asymmetrical Calcium transients influence left-right patterning is to be investigated in more detail (Mizuno et al., 2020). Particularly, it has been suggested that Calcium transients activate RNA binding protein Bicc1 which in turn suppresses the translation of nodal antagonist *dand5* mRNA (Maerker et al., 2021). The LRO is formed by axial and (in *Xenopus*) paraxial mesoderm progenitors which form the gastrocoel roof plate in amphibian (Shook et al., 2004; Blum et al., 2009), the posterior notochord in mouse and rabbit (Schroder et al., 2016; Blum et al., 2007) or the Kupffer’s vesicle in zebrafish embryos (Essner et al., 2005). Function of LRO requires the correct domain architecture and differentiation. The effectively directed flow was suggested to be caused by posterior position and structural features of the motile cilia (Nonaka et al., 2005). Hence, the correct position of cilia is a function of established planar cell polarity along anterior-posterior axis (Antic et al., 2010; Hashimoto and Hamada, 2010; Hashimoto et al., 2010; Song et al., 2010; May-Simera et al., 2010; Borovina et al., 2010). In *Xenopus* and mouse embryo LRO includes spatially segregated domains characterized by gene expression and types of cilia (Schweickert et al., 2010; Blum et al., 2009; Petri et al., 2024). Particularly, lateral domains which form at both sides immotile cilia bilaterally express *nodal*, its antagonist *dand5* and in *Xenopus* somitic marker *myoD* (Schweickert et al., 2010; Petri et al., 2024; Tingler et al., 2022). Importantly, to create the appropriate flow and detect it, ciliated cells should be transiently faced towards a cavity of the LRO whereas after the symmetry breaking event, the surface is covered by endodermal cells. Abnormal development of LRO with a LRO covered by endoderm prevents correct symmetry breaking in *Xenopus* (Schneider et al., 2019; Petri et al., 2022). Recent observations indicate involvement of morphogen transport in left-right symmetry breaking (Ott et al., 2025; Lee et al., 2024) suggesting a complex and possibly double-secured mechanism. Flow-mediated transport of PKD to the left of the mouse LRO has been suggested to contribute to left-right symmetry breaking in the mouse embryo (Tanaka et al., 2023). It must be mentioned that further, earlier asymmetries have been observed in *Xenopus* and mechanisms based on these have been discussed (Onjiko et al., 2021; Onjiko et al., 2016; Vandenberg and Levin, 2013), which still have to be integrated into the existing models.

There is another way

Leftward flow has been suggested to be required for directed LR symmetry breaking in many vertebrate models (Blum and Ott, 2018; Hamada, 2020; Blum et al., 2014) and is one of the most fascinating observations in developmental biology. However, structural elements required for leftward flow are absent in several model organisms. Structural units required for ciliary flow were not detected in chick and pig embryos where the ventral surface of axial mesoderm was shown to be covered by endoderm and subchordal mesoderm (Manner, 2001; Gros et al., 2009) while analysed

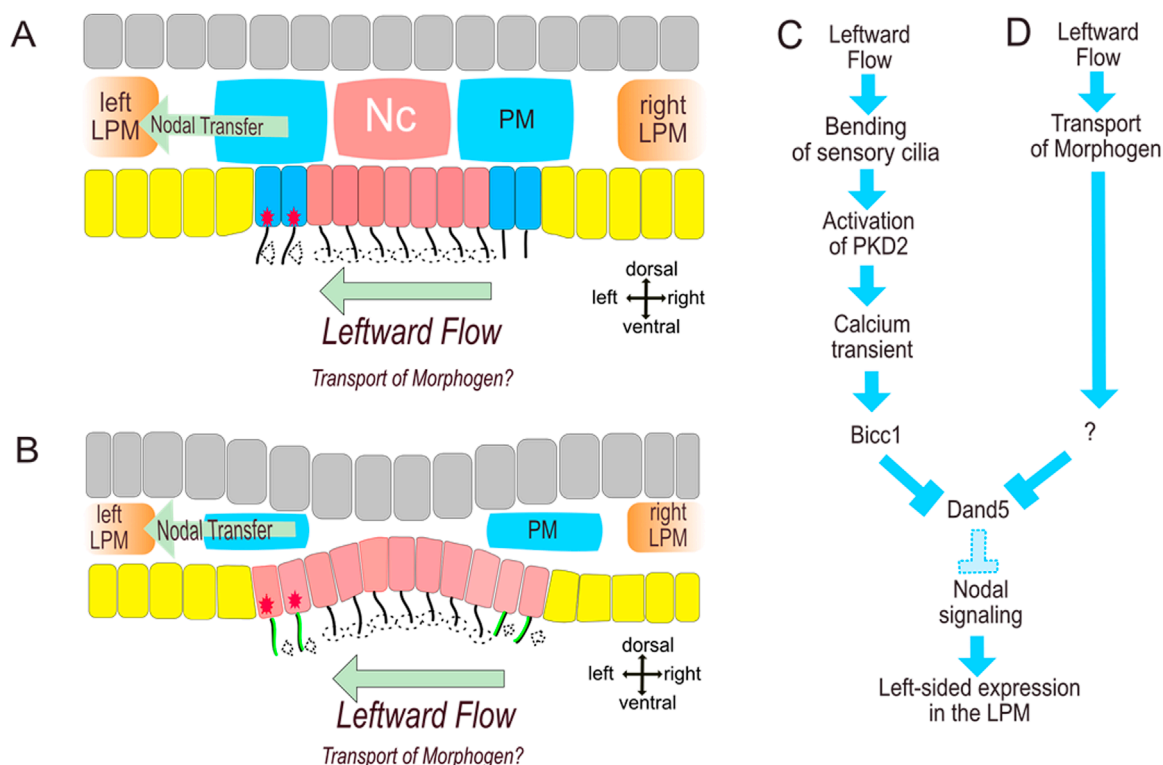


FIGURE 1

Left-Right-Organizer and Symmetry breaking in *Xenopus* (A) and Mouse (B). (A,B) morphology, (C) important steps of two-cilia model, (D) proposed transport of morphogens. Clockwise rotation of monocilia in the midline of gastrocoel roof plate (A) or posterior notochord (B) creates leftward flow which causes deflection of sensory cilia formed by superficial somitic progenitor cells. Deflection mediates Calcium transients (red stars) and further processes leading to left-sided nodal activation in the paraxial and later in the lateral plate mesoderm (C). Yellow: endoderm, grey: ectoderm, red: axial mesoderm, dark blue: superficial somitic mesoderm, blue: deep somitic mesoderm, LPM: lateral plate mesoderm. PKD localization (green) in the medial (dorsal) segment in mouse immotile cilia (B) ensures left-sided activation despite the deflection of the right-sided sensory cilia to the left (dashed arrows at the right side of the LRO).

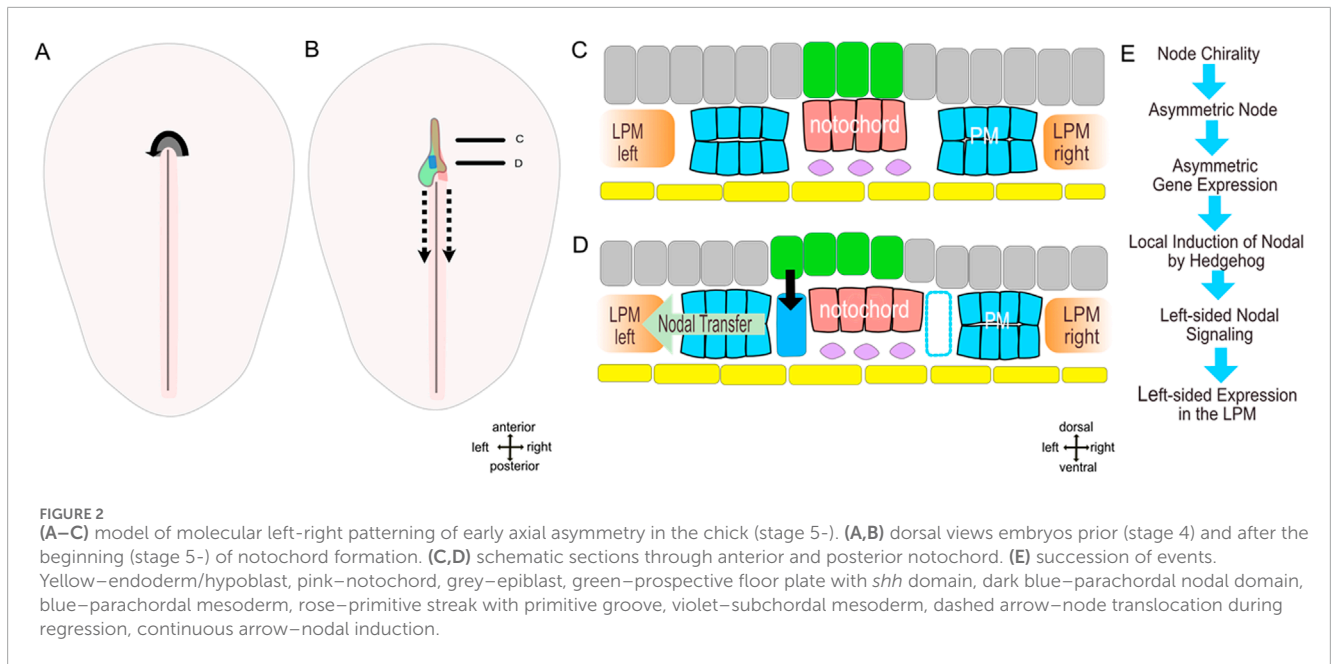
non-avian reptilian do not form motile cilia (Kajikawa et al., 2020; Shylo et al., 2023). Furthermore, in the chick, the first signs of left-right asymmetry occur at late gastrula stage and include the morphological asymmetry of the node and asymmetric leftward cell movements around the node (Dathe et al., 2002; Cui et al., 2009). These observations also match the asymmetric gene expression in the chick node prior to asymmetry of *nodal* expression in the lateral plate mesoderm: *sonic hedgehog* morphogen (*shh*) was shown to be expressed asymmetrically in the node of notochord stage (stage 5) chick embryo (Levin et al., 1995; Pagan-Westphal and Tabin, 1998) while this asymmetry was suggested to cause left-sided *nodal* expression at somitogenesis stages. Further analysis of chicken node suggested asymmetrical morphology of the node itself prior to asymmetric gene expression (Dathe et al., 2002).

We suggest that the chick primitive node plays the role of the LRO. In the following we explain the corresponding developmental context.

This is the way (of the chicken)

Gastrulation in the chick starts with the formation of the primitive streak in the posterior area of circle-shaped bilayered

embryonic disc (stage 1). Cell intercalation in the posterior epiblast has been suggested as a cellular mechanism of primitive streak formation. Intercalation was shown to be PCP-dependent (Voiculescu et al., 2007) and/or be based on Myosin-II based contractions of epiblast cell groups (Rozbicki et al., 2015). Cell division in the anterior pole of the embryo accounts for the tissue fluidity that is necessary for primitive streak formation (Firmino et al., 2016) while the tensile ring at the margin of the *area pellucida* is required for tissue flow during primitive streak formation (Saadaoui et al., 2020). Primitive streak undergoes elongation which is accompanied by bilateral flow of cells of dorsal (epiblast) layer towards the streak. The node which gives rise to the axial mesoderm emerges at the anterior tip of the streak and starts to form the prechordal mesoderm (Stern and Stern, 2004). The maximum of elongation of the primitive streak is followed by the beginning of notochord formation and concomitant shortening of the streak also referred to as streak regression. Global cell movements towards the emerging streak reveal left-right asymmetry, even if the functional significance of this asymmetry remains to be tested (Asai et al., 2025). The first morphological asymmetry arises in the node at the beginning of notochord formation: dorsal views reveal that the right edge of the node is thicker than the left edge while sections demonstrate that this



difference is due to mesodermal density at the right side below the epithelial epiblast (Tsikolia et al., 2012). Asymmetric position of density is already detectable prior to the emergence of the notochord. Emergence of the asymmetric notochord is preceded by asymmetrical counterclockwise cell movements within the node (cf. Supplementary Figure). It was suggested that the progressive asymmetry of *sonic hedgehog* expression is due to displacement of *shh* domain to the left-side while pharmacological evidence indicates that node rotation requires actomyosin contractility and activity of ATP4a proton pump (Gros et al., 2009). Moreover, N-Cadherin is involved in termination of node rotation (Mendes et al., 2014). Further analysis however, revealed, that paraxial asymmetrical expression of *nodal* is initiated at the beginning of notochord formation while *shh* is still expressed at both sides of the node (Tsikolia et al., 2012) hence indicating that *nodal* asymmetry is not a direct effect of node and *shh* domain rotation. Asymmetry of the node and asymmetric *shh* expression increase concomitant with node regression during stages 5 and 6. Surprisingly *shh*-expression at these stages is confined to the epiblast of the left node shoulder, to the floor plate, to the prechordal mesoderm but not to the posterior notochord (Kremnyov et al., 2018) challenging the widely assumed induction of floor plate by the notochord.

Morphological analysis of the node and notochord reveals the continuity of the notochord with the right node shoulder. Assuming that notochord progenitor cells are generated within the node this continuity suggests an asymmetrical notochord formation. This proposal is supported by analysis of notochordal marker expression particularly *noggin* (Otto et al., 2014) and *brachyury*. The origin of the notochord from the right side of the node results in positioning of the *shh* expressing floor plate to the left of the notochord and immediately above the paraxial *nodal* expressing area. This spatial proximity suggests a local induction of the paraxial *nodal* domain by hedgehog signaling activated by *shh* ligand secreted from the floor plate. Indeed, the inhibition of

hedgehog signaling leads to an absent paraxial *nodal* expression (Otto et al., 2014) while ectopic hedgehog activation (Negretti et al., 2022) causes bilateral *nodal* expression. This data strongly suggests that secreted hedgehog is both necessary and sufficient for paraxial *nodal* induction in the chick while the asymmetry of *nodal* is due to asymmetry of the notochord formation (Figure 2). Interestingly, prior to asymmetric morphogenesis the node revealed matrix-filled spaces which may be involved in regulation of molecular activity (Pieper et al., 2020).

Cytoskeleton: hidden egg?

What are the mechanisms leading to the asymmetric morphogenesis of the avian node? Leftward rotation of the node is a robust morphogenetic event and indicates intrinsic tissue chirality which in turn has been suggested to have roots in the cytoskeletal organisation particularly in chiral features of the cytoskeletal constituents such as microtubules or actin (Wan and Vunjak-Novakovic, 2011; Xu et al., 2007; Yamanaka and Kondo, 2015). Indeed, cytoskeletal asymmetries were shown to be involved in left-right symmetry breaking during development of *C. elegans* which reveals chiral cortical flow related to contractility of actomyosin (Naganathan et al., 2014) and regulated by RhoA and Cyk1/Formin (Middelkoop et al., 2021; Pimpale et al., 2020). Chiral development of snails is also controlled by formins *dia1* and *dia2* (Abe and Kuroda, 2019) which regulate actin nucleation and polymerisation while further actin nucleator DAAM (Chougule et al., 2020) together with unconventional myosin ID (Myo1D) are involved in left-right asymmetry in *Drosophila melanogaster* (Coutelis et al., 2008; Speder et al., 2006). Similar to other members of Myosin I family, Myosin ID was shown to link actin with membrane lipids (McAlpine et al., 2018) and it has been suggested that the role of Myosin ID as a chiral determinant is due to its chiral interaction with actin (Juan et al., 2018).

What's next?

It has been proposed that cellular-scale chirality arises from chiral structures by interaction within “chirality modules” particularly between actin filaments and formin dimers, whereas the understanding of transition to tissue chirality remains challenging (Tsikolia et al., 2025).

How does the cellular chirality contribute to left-right symmetry breaking in the chick? At elongated streak stage only the node tissue displays chiral behaviour which manifests itself during a short period of time. This indicates that the supracellular manifestation of chirality is a subject of regulation. The node which is equivalent of Spemann's organizer (Stern and Stern, 2004) undergoes different stages: it emerges in the area expressing organizer genes, contributes to the prechordal mesoderm, endoderm, medial paraxial and axial mesoderm. Node rotation takes place after migration of the prechordal mesoderm and clustering of axial mesoderm progenitors in the node mesoderm (Tsikolia et al., 2012). These events may stimulate activation of tissue chirality in the node area. Whether this activation is due to specific molecular pathway or structural constraint has to be investigated in the next step.

Author contributions

TP: Writing – original draft, Writing – review and editing. NT: Conceptualization, Visualization, Writing – original draft, Writing – review and editing.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcell.2025.1672263/full#supplementary-material>

SUPPLEMENTARY FIGURE

Asymmetrical node morphogenesis in the chick embryo (A–C) scanning electron microscopy of the node prior to notochord formation (stage 4: A,B) and at the early notochord stage (stage 5, C,D) embryos. (B) schematic view of asymmetrical node (green) displacement (as reported by Gros et al., 2009 and Cui et al., 2009), (C)– emergence of thicker right node shoulder (rs). (D) floor plate (green) and notochord (red) domains indicated at the surface. A modified from Figure 1F (Tsikolia et al., 2012) with permission.

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