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Cell movements at Hensen's node establish Left/Right asymmetric gene expression in the chick

Jerome Gros[#], Kerstin Feistel^{*,^}, Christoph Viebahn[±], Martin Blum^{*}, and Clifford J. Tabin[#],
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[#] Department of Genetics, Harvard Medical School, Boston MA 02115

^{*} Institute of Zoology, Hohenheim University, 70953 Stuttgart, Germany

[±] Department of Anatomy and Embryology, Göttingen University, 37079 Göttingen, Germany

Abstract

In vertebrates, the readily apparent left-right (L/R) anatomical asymmetries of the internal organs can be traced to molecular events initiated at or near the time of gastrulation. However, the earliest steps of this process do not seem to be universally conserved. In particular, how this axis is first defined in chicks has remained problematic. Here we show that asymmetric cell rearrangements take place within chick embryos, creating a leftward movement of cells around the node. It is the relative displacement of cells expressing Sonic hedgehog (Shh) and Fibroblast growth factor 8 (Fgf8) that is responsible for establishing their asymmetric expression patterns. The creation of asymmetric expression domains as a passive effect of cell movements represents an alternative strategy for breaking L/R symmetry in gene activity.

In mice and rabbits monocilia responsible found on cells of the posterior notochordal plate have been shown to play a crucial role in breaking L/R symmetry (1,2). These cilia are able to create a leftward flow of fluid, in a pit-like teardrop shaped space that is not covered by subjacent endoderm (3)., The flow of fluid across this pit stimulates signal transduction that ultimately leads to induction of asymmetric gene expression (1,2).

In the chick embryo, in contrast, the endoderm underlying Hensen's node (a structure at the rostral end of the primitive streak in the gastrulating embryo) exists as a continuous sheet ventral to the notochordal plate mesoderm (4) and there is no morphological pit on the ventral surface in which a flow of fluid could be established. Prior work has noted cilia at Hensen's node (5) but these short cilia are on endodermal cells and are unrelated to the motile cilia on the mesodermal cells of the ventral node in the mouse and rabbit. The mesodermal cells at Hensen's node in the chick are devoid of cilia. In addition, the Talpid chick mutant lacks primary cilia (6) but does not exhibit L/R asymmetry defects. Unlike the mouse and rabbit, the chick node itself becomes morphologically asymmetric, with a marked tilt towards the left around the time the primitive streak reaches full extension, at Stage 4. (7,8) (Fig. 1A-C). Shortly thereafter, a number of small L/R asymmetric expression domains are observed to the right and left of the node (9). However, all of the genes expressed in such a manner are initially expressed in a symmetric fashion, for example, Fgf8 bilaterally along the primitive streak and Shh bilaterally across the top of the node until stage 4, (10) (Fig. 1D_G). Subsequently, concomitant with the development of

⁺To whom correspondence should be addressed: Phone: 617-432-7799, Fax: 617-432-7595, tabin@genetics.med.harvard.edu.

[^]Current address: Division of Neuroscience, Oregon National Primate Research Center, Oregon Health and Science University, Beaverton OR 97006

morphological asymmetries in the node, these gene expression patterns also become gradually asymmetric by stage 5 (Fig. 1H,I).

To investigate cellular rearrangements that could be responsible for establishing the morphological asymmetry of the node, we performed a time lapse analysis of cell movements at Hensen's node; randomly labeling cells by electroporation of a green fluorescent protein (GFP) reporter. At stage 4, as primitive streak elongation terminates (Fig. 1K, O) but regression has not yet begun (Fig. 1M, Q), the cells that have formed the node exhibit a definitive, albeit brief (3–4 hours) leftward movement (n=10/10, Movies S1,S2,S3 and Fig. 1L, P).

We next attempted to block the leftward movement of cells at the node by the use of drugs that inhibit the function of Rho Kinase (Rok) (Y-27632) and its target MyosinII (Blebbistatin). These two proteins have been shown to provide the force driving various oriented cell rearrangements in several species. After adding these drugs, gastrulation movements continued (Fig. 1R, Fig. S1B,F,J) and most reached at least stage 6, when the notochord has formed, although as previously described (11,12) primitive-streak regression was affected (Fig. 1T, Fig. S1D, H, L). In these drug-treated embryos, the leftward movements of cells at the node was no longer observed (Movie S4, S5 and Fig. 1S, Fig. S1C,G,K). 3D reconstruction of Hensen's node from stage 5 embryos that were cultured in Blebbistatin or Y-27632 revealed that the node displays a more symmetrical and anteriorly extended shape as compared to control embryos (dotted lines, Fig. 2A-B, E-F, Fig. S2A-B), suggesting that the cell movements we identified are responsible for the morphological asymmetry of the node. We moreover noted that these embryos also displayed symmetrical expression of Shh and FGF8 (Y-27632 n= 16/23 and Blebbistatin n=17/21, Fig. 2G-H, Fig. S2C-D).

We additionally attempted to block the leftward cell movements anterior to the node by two physical methods: manually inserting individual human hairs through the primitive streak extending anterior to the node and surgically bisecting the embryos along the midline. Both manipulations also led to bilateral gene expression (Fig. S3). Thus the leftward cellular movements at the node are necessary to initiate L/R asymmetric expression domains in the chick.

It has been previously demonstrated that asymmetric expression of Shh and FGF8 depends on a transient, H⁺/K⁺ ATPase-dependent depolarization of membrane potential on the left side of the primitive streak, just before stage 4 (13). We cultured embryos in plates containing the H⁺/K⁺ ATPase inhibitors, SCH28080 or Omeprazole, and found that the node displayed a symmetrical morphology comparable to the phenotypes observed with MyosinII and Rok inhibitors, suggesting that in these conditions the cell rearrangements at the node did not occur properly (Fig. 2I-J, Fig. S2E-F). Moreover, when we performed a time lapse analysis on GFP electroporated embryos cultured on plates containing Omeprazole, we did not observe any leftward movements at the node (n=3/4) (Movie S6 and Fig. 1V, Fig. S1O) as observed in control embryos (movie 1 n=10/10 Fig. 1L, P), although the primitive streak elongated and regressed normally (Fig. 1U-W, Fig. S1N-P). Embryos treated with H⁺/K⁺ ATPase inhibitors exhibited symmetrical expression of Shh and FGF8 (as previously reported (13)), by stage 5–6 (SCH28080 n=7/12 and Omeprazole n= 6/7, Fig. 2K,L, Fig. S2G-H). These data show that the leftward movement at the node is downstream of the asymmetric H⁺/K⁺ ATPase activity.

The mechanism for setting up L/R asymmetric gene expression patterns at the chick node may have relevance beyond avian species. Despite the morphological conservation of the notochordal plate's ventral surface as seen in mice, rabbits and, possibly, humans (14),

histological analysis reveals that, similar to the chick, the cells of the pig notochordal plate do not contain cilia (Fig. 3A-C, Fig. S4D-J). As in the chick, we do not observe any structure in the pig resembling the ventral node of the mouse and rabbit. Moreover, as in the chick, the cells of the pig notochordal plate are completely covered by endoderm. As a consequence, there is no space in which a flow of fluid could be generated (Fig. 3E, Fig. S4A, B). Additionally, like the chick, pig embryos have a morphologically asymmetric node displaced leftward at Stage 5 (n=4/4) (Fig. 3C), and like the chick and unlike the mouse, there are asymmetric gene expression domains adjacent to the node, such as *Foxj1* (n=3/3) (Fig. 3A), which precede by several hours the asymmetric expression of *Nodal* at Stage 6 (Fig. 3D) (*Nodal* being the first gene known to be asymmetrically expressed in mice and rabbits).

In summary we show here that, in chick embryos, the node is the site of cellular rearrangements that create a leftward movement of cells around it (an observation made independently by Cui et al (15)). The convergence of cells on the right edge of the node and migration away from the midline on the left thereby deform the shape of the node. This movement establishes asymmetric gene expression patterns, not through transcriptional induction or repression but rather in a passive manner by rearranging the relative orientations of cells expressing critical genes (Fig. S5). Moreover, we have found that cell movements at the node arise downstream of a transient depolarization of membrane potential on the left side mediated by the activity of the H⁺/K⁺ ATPase. The symmetry-breaking event that leads to asymmetric H⁺/K⁺ ATPase activity in chick remains unknown.

Previous mis-expression experiments have put the genes asymmetrically expressed at the chick node into an epistatic pathway. For example, according to current models, *Bmp4* (which is expressed in very similar domains to *Fgf8*, initially bilaterally along the streak and then asymmetrically on the right side) induces *Fgf8* and represses *Shh*. *Shh* feeds back to repress *Bmp4* and induce *Nodal*, whereas *Fgf8* serves to repress *Nodal*. Our data do not contradict these previously described epistatic relationships. However, since the asymmetric expression domains of these signaling molecules do not form in the absence of cell movements, we suggest that the previously described cross-regulation, for instance the reciprocal inhibition between *Shh* and *Bmp4*, functions secondarily to sharpen borders and provide robustness once cells expressing these factors are brought into juxtaposition, rather than as a primary means of establishing their asymmetric gene expression domains.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References and Notes

1. Levin M. Left-right asymmetry in embryonic development: a comprehensive review. *Mech Dev* 2005;122(1):3–25. [PubMed: 15582774]
2. Raya A, Belmonte JC. Left-right asymmetry in the vertebrate embryo: from early information to higher-level integration. *Nat Rev Genet* 2006;7(4):283–293. [PubMed: 16543932]

3. Lee JD, Anderson KV. Morphogenesis of the node and notochord: the cellular basis for the establishment and maintenance of left-right asymmetry in the mouse. *Dev Dyn* 2008;237(12):3464–3476. [PubMed: 18629866]
4. Kirby ML, et al. Hensen's node gives rise to the ventral midline of the foregut: implications for organizing head and heart development. *Dev Biol* 2003;253 (2):175–188. [PubMed: 12645923]
5. Essner JJ, et al. Conserved function for embryonic nodal cilia. *Nature* 2002;418(6893):37–38. [PubMed: 12097899]
6. Yin Y, et al. The *Talpid3* gene (KIAA0586) encodes a centrosomal protein that is essential for primary cilia formation. *Development* 2009;116:655–664. [PubMed: 19144723]
7. Hertwig, O. *Lehrbuch der Entwicklungsgeschichte des Menschen und der Wirbelthiere*. Vol. 7. Aufl. Fischer; Jena: p. 1902
8. Kölliker, A. *Entwicklungsgeschichte des Menschen und höheren Thiere*. Wilhelm Engelmann; Leipzig: 1879.
9. Dathe V, et al. Morphological left-right asymmetry of Hensen's node precedes the asymmetric expression of *Shh* and *Fgf8* in the chick embryo. *Anat Embryol (Berl)* 2002;205(506):343–354. [PubMed: 12382138]
10. Levin M, et al. A molecular pathway determining left-right asymmetry in chick embryogenesis. *Cell* 1995;82(5):803–814. [PubMed: 7671308]
11. Marlow, et al. Zebrafish Rho kinase 2 acts downstream of *Wnt11* to mediate cell polarity and effective convergence and extension movements. *Curr Biol* 2002;12(11):876–884. [PubMed: 12062050]
12. Wei L, et al. Rho kinases play an obligatory role in vertebrate embryonic organogenesis. *Development* 2001;128(15):2953–2962. [PubMed: 11532918]
13. Levin M, et al. Asymmetries in *H⁺/K⁺-ATPase* and cell membrane potentials comprise a very early step in left-right patterning. *Cell* 2002;111(1):77–89. [PubMed: 12372302]
14. O'Rahilly, R.; Muller, F. *The embryonic human brain: an atlas of developmental stages*. 3. Wiley-Liss; 2006.
15. Cui C, Little C, Rongish B. Rotation of organizer tissue contributes to left-right asymmetry. *Anat Rec (Hoboken)* 2009;294(4):557–561. [PubMed: 19301278]

Fig. 1.

Morphological and molecular asymmetries arise in conjunction with a leftward movement around the chick Hensen's node at stage 4 (A-C) 3D reconstruction of confocal views (Z-stack) of Phalloidin stained embryos at stage 3 (A), 4 (B) and 5 (C). Most dorsal ("D") staining is depth coded blue; the most ventral ("V") staining is red. (D-I) *In situ* hybridizations for Shh (D, F, H) and FGF8 (E, G, I). Red arrows, asymmetric domain of Shh; yellow arrows, asymmetric domain of Fgf8. (J-M) Time series showing movement of electroporated cells. (J) Embryos were electroporated at Stage 3+, and subsequent time points are indicated in the lower right corner. 10–30% of cells are labelled by GFP. Note: cells undergo a leftward movement around the node (L). White arrows (N-Q) show the trajectories of the cells shown in (J-M, respectively). In blebbistatin treated embryos (R-T), early cell movements are relatively unaffected, however there is no leftward movement around the node (S). Later, cells display disorganized movements and the primitive streak fails to regress (T). In Omeprazole treated embryos (U-W) gastrulation movements are normal, however cells from the left and right sides move symmetrically toward the node but not around it. The position of the streak and of the node pit are noted by a red-dotted line and a red circle, respectively.

Fig. 2.

Effects of MyosinII and voltage gradient inhibitors on node morphology and asymmetric expression domains.

(A, E, I) 3D reconstruction of confocal views (Z-stacks) of stage 5 embryos show the morphology of the node and the primitive streak. Lower panels represent a virtual cross section at the level of the white-dotted line. (B, F, J) Depth-coded 3D reconstruction of confocal views (Z-stack) of Phalloidin staining; the dorsal-most staining is blue, the ventral-most is red. The shape of the node is outlined by a white-dotted shape. *In situ* hybridization of Shh (C, G, K) and FGF8 (D, H, L). Red arrow, extent of posterior Shh expression; yellow arrow, extent of anterior Fgf8 expression.

Fig. 3.

Similarities between the gastrulation node in the pig embryo and Hensen's node during chick gastrulation.

(A–C) Ventral scanning electron microscopy views of a 5–6 somites stage pig embryo with higher magnifications of black box in (B,C) of the notochordal plate and adjacent paraxial mesoderm. The endoderm forms a continuous ventral cover (that carries short stubby cilia of 1.5 μ m length, red arrowheads) across the entire ventral surface of the embryo. (D) A sagittal section at the level of the presumptive floor plate (fp) and notochord (no), covered by a continuous layer of endoderm (end). Anterior direction indicated by ant., leftward facing arrow. (E) The asymmetric node of a Stage 5 pig embryo, marked by asymmetric expression (arrow heads) of the transcription factor Foxj1, demonstrating a shift of the primitive pit (pp, stippled blackline). R, right; L, left. For more information see Fig. S4.