DEVELOPMENTAL BIOLOGY

Cilia Discern Left from Right

Determination of vertebrate left-right body asymmetry requires immotile cilia that sense fluid flow generated by nearby motile cilia.

Dominic P. Norris and Daniel T. Grimes

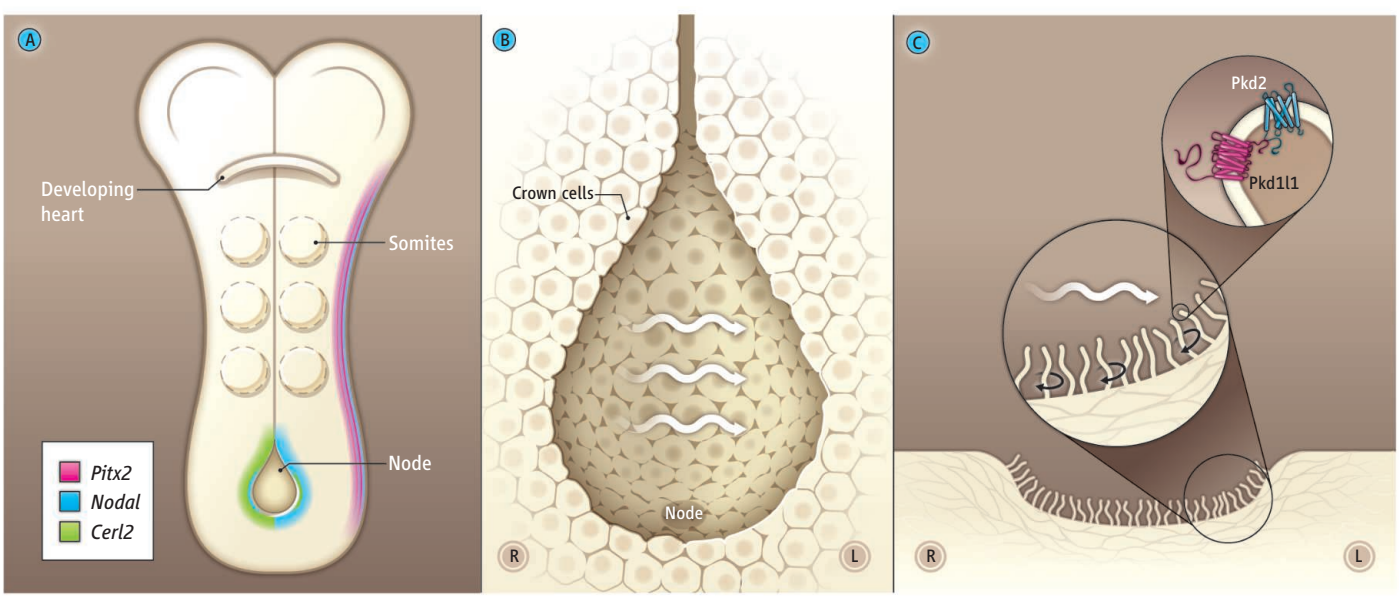
Although the human body shows left-right (L-R) mirror symmetry when viewed externally, the placement and patterning of the internal organs and associated vasculature are strikingly asymmetrical. In the mammalian early embryo, L-R symmetry is broken by the action of rotating cilia—small hair-like protrusions on the surface of cells—in a pit-like structure called the node (see the figure).These cilia generate a unidirectional flow of fluid across the node from right to left (*1*). This nodal flow breaks L-R symmetry by driving asymmetries in gene expression and Ca^{2+} signaling in cells at the periphery of the node. On page 226 of this issue, Yoshiba *et al*. (*2*) reveal a mechanism by which the mouse embryo senses nodal flow.

Surrounding the node pit with its rotating cilia are crown cells bearing immotile cilia (*3*). A long-standing model, the "two-cilia" hypothesis, argues that the peripheral immotile cilia sense the flow generated by the centrally located motile cilia (*3*, *4*). The asymmetric signals that are induced by flow are first apparent in the crown cells. Here, the secreted signaling molecule Nodal is expressed more strongly on the left than on the right, whereas its antagonist, the secreted molecule Cerberus-like 2 (Cerl2), is expressed with the opposite bias. Subsequently, Nodal and its target, the gene encoding the transcription factor Paired-like homeodomain transcription factor 2 (Pitx2), are expressed more laterally along the left but not right side of the embryo. Although asymmetric Nodal is only briefly expressed, the expression of left-sided Pitx2 is maintained longer and controls asymmetric organ patterning. The link between the asymmetric gene expression in the cells surrounding the node and the flow of fluid across the node from right to left has, however, remained unresolved.

The transmembrane cation channel Polycystin-2 (Pkd2) is required for L-R patterning in the vertebrate embryo and is implicated in the asymmetric elevation in intracellular calcium concentration ("Ca^{2+} spike") that occurs to the left of the node in response to flow (*3*, *5*). The mouse *Pkd2* gene is ubiquitously expressed, making its site of action uncertain. Yoshiba *et al*. analyzed *Pkd2*-deficient mutant mouse embryos. By rescuing expression of the gene in only specific regions—the entire node (crown and pit cells), crown cells only, or solely pit cells—the authors demonstrate that *Pkd2* expression in node pit cells is not sufficient to rescue L-R development. By contrast, expression of *Pkd2* in just the crown cells rescued normal L-R development, demonstrating that *Pkd2* is specifically required in these peripheral cells, where most immotile cilia are located. Pkd2 protein normally localizes to cilia in the node (*3*), suggesting that its role during the breaking of L-R symmetry is played within cilia. The *Pkd2*^{lrm4} mutant mouse (*6*) exhibits severe L-R defects similar to those of *Pkd2*-deficient mice (*5*). Yoshiba *et al*. show that although the mutant Pkd2^{lrm4} protein retains normal Ca^{2+} channel activity, it fails to localize to cilia within the node, supporting the idea that Pkd2 normally acts in cilia. Asymmetry of *Cerl2* gene expression, which is stronger on the right than on the left side of the node, depends on Pkd2 (*7*); Yoshiba *et al*. go further, demonstrating that *Cerl2* is a target of flow-induced Pkd2-mediated signals.

Because Pkd2 is a Ca^{2+} channel and a Ca^{2+} spike around the node is stronger on the left than on the right (*3*), it has been thought that Pkd2, acting downstream of flow, elicits this Ca^{2+} asymmetry. However, by imaging Ca^{2+} specifically in crown cells, Yoshiba *et al*. make the surprising finding that Ca^{2+} concentrations show no L-R differences in these cells. Intriguingly, through pharmacological

MRC Mammalian Genetics Unit, Oxfordshire OX11 0RD, UK. E-mail: d.norris@har.mrc.ac.uk



Feel the flow. (**A**) The early mouse embryo (day 8.25), showing asymmetric expression of the indicated genes around the node and laterally. (**B**) The node consists of a depression containing central pit cells with motile cilia and peripheral crown cells with immotile sensory cilia. (**C**) Cross section through the node showing Pkd1l1-Pkd2 complexes in crown cell cilia sensing leftward flow. The signaling pathway links fluid flow across the node with asymmetric gene expression.

CREDIT: Y. HAMMOND/*SCIENCE*

Published by AAAS

intervention, the authors demonstrate a role for Ca^{2+} signaling in L-R asymmetry within crown cells. Thus, the links between Ca^{2+} signaling in crown cells and L-R asymmetry are still to be clarified. The asymmetrical Ca^{2+} signal (*3*) is likely occurring in more lateral endoderm cells, where it may play a role in the transfer of asymmetric signals from the node to the left side of the embryo (*8*).

Cilia are sensory organelles (*9*). In kidney epithelial cells, where Polycystin-1 (Pkd1) and Pkd2 localize to cilia, they act as flow sensors (*10*). *Kif3a* is required for cilia formation, and *Kif3a*-deficient mutant mice lack all node cilia (*11*, *12*). When expression was rescued only in crown cells, the resulting mouse embryos lacked the central motile cilia, but still possessed the putative sensory cilia around the node periphery. Application of exogenous rightward fluid flow resulted in activation on the right side of the normally left-restricted genes. Thus, the presence of immotile cilia on the crown cells is sufficient for sensing flow in the node, strongly supporting the two-cilia hypothesis.

The results of Yoshiba *et al*. suggest a model in which Pkd2, acting within crown cell cilia, is part of the leftward flow-sensing unit and that flow activates Pkd2, which in turn inhibits expression of *Cerl2*. However, Pkd2 is thought not to be a sensory protein but instead part of a complex with a sensory partner, such as Pkd1 in the kidney. A strong flow-sensing candidate protein, recently identified as critical in L-R determination in mouse and fish, is the polycystin transmembrane protein polycystic kidney disease 1-like 1 (Pkd1l1) (*7*, *13*). Pkd1l1 forms a complex with Pkd2 in cilia (*7*, *13*), leading to the notion that the flow-sensing complex comprises the sensor Pkd1l1 and the effector Pkd2.

How do the crown cell cilia sense flow? One possibility is through chemosensation of a determinant molecule that becomes enriched on the left in response to flow; another is by direct mechanosensation of the force induced by flow itself. No experiment has yet distinguished these possibilities, though circumstantial evidence supports the mechanosensation hypothesis. By analogy to Pkd1 function in kidney, Pkd1l1 might mediate mechanosensation in the early embryo (*7*). Indeed, mutation of a region in the extracellular portion of both Pkd1l1 and Pkd1 (called the PKD domain) abrogates function, consistent with the role of the domain in force detection (*7*, *14*). However, the lack of detectable immotile cilia in the medaka fish Kupffer's vesicle—the functional homolog of the mouse node—argues against mechanosensation (*13*). Furthermore, the left-sided enrichment of a chemical in the node is physically possible (*15*). A broad approach encompassing genetics, embryology, and biophysics, likely in combination with an understanding of microfluidics and physical modeling, will be required to unravel the possibilities.

References and Notes

1. S. Nonaka *et al*., *Cell* **95**, 829 (1998).
2. S. Yoshiba *et al*., *Science* **338**, 226 (2012); 10.1126/science.1222538.
3. J. McGrath, S. Somlo, S. Makova, X. Tian, M. Brueckner, *Cell* **114**, 61 (2003).
4. C. J. Tabin, K. J. Vogan, *Genes Dev.* **17**, 1 (2003).
5. P. Pennekamp *et al*., *Curr. Biol.* **12**, 938 (2002).
6. A. Ermakov *et al*., *Dev. Dyn.* **238**, 581 (2009).
7. S. Field *et al*., *Development* **138**, 1131 (2011).
8. M. Viotti, L. Niu, S. H. Shi, A. K. Hadjantonakis, *PLoS Biol.* **10**, e1001276 (2012).
9. V. Singla, J. F. Reiter, *Science* **313**, 629 (2006).
10. S. M. Nauli *et al*., *Nat. Genet.* **33**, 129 (2003).
11. S. Takeda *et al*., *J. Cell Biol.* **145**, 825 (1999).
12. J. R. Marszalek, P. Ruiz-Lozano, E. Roberts, K. R. Chien, L. S. Goldstein, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 5043 (1999).
13. K. Kamura *et al*., *Development* **138**, 1121 (2011).
14. L. Ma, M. Xu, J. R. Forman, J. Clarke, A. F. Oberhauser, *J. Biol. Chem.* **284**, 32942 (2009).
15. Y. Okada, S. Takeda, Y. Tanaka, J.-C. I. Belmonte, N. Hirokawa, *Cell* **121**, 633 (2005).

Acknowledgments: D.P.N. and D.T.G. are supported by the UK Medical Research Council.

10.1126/science.1230401

GEOPHYSICS

Seeing Is Believing

Benjamin A. Brooks

Satellite data and computational modeling provide evidence for a spheroidal magma body rising within the crust below the Altiplano Plateau in the central Andes.

The lava lamp on my son's bureau gives him a vantage point to ponder the colorful blobs rising and falling as their temperature and density change. Geologists are not so privileged. Some of Earth's most impressive geologic features, such as the massive granitic plutons of California's Sierra Nevada mountains, are direct consequences of magma transport but we cannot directly observe these transport processes. On page 250 of this issue, Fialko and Pearse (*1*) use satellite data and computational modeling to infer the transport process in one location. They conclude that a magmatic diapir—a roughly spheroidal mass of partially molten material—is rising beneath a portion of the Altiplano Plateau in the central Andes.

School of Ocean and Earth Science and Technology, University of Hawaii, Honolulu, HI 96822, USA. E-mail: bbrooks@hawaii.edu

To deduce subsurface processes, geologists must compare displacements of millimeters to centimeters per year on Earth's surface with mathematical models. However, different models can give similar predictions within the observational errors, and the most physically plausible models are often too computationally intensive to allow adequate comparison with the data. Fundamental advances thus require some combination of increased observational and computational power. In their tour-de-force study, Fialko and Pearse achieve both.

The authors used the interferometric synthetic aperture radar (InSAR) technique (*2*) with data from three different satellites to create an 18-year record of surface displacement at Bolivia's Uturuncu volcano. The study region sits directly above what is believed to be the largest active magma body in Earth's continental crust: the Altiplano-Puna Ultralow-Velocity Zone (APULVZ), which is ~17 to 19 km deep, ~1 km thick, and ~100 km wide (*3*, *4*).

Previous studies, based on 8 years of InSAR data from two satellites with similar viewing geometry, identified surface uplift associated with Uturuncu and modeled it as the manifestation of an inflating magmatic point source at a depth of ~15 to 17 km (*5*). To better constrain the depth, geometry, and time history of the supposed magmatic source, Fialko and Pearse tasked a third satellite to acquire data from a very different look direction. Together with the previously acquired data, this more robust data set revealed an unexpected pattern of subsidence around the periphery of uplift.

The combined uplift and subsidence is difficult to explain with models that assume elastic behavior of Earth's crust. Fialko and Pearse therefore tested increasingly sophisticated viscoelastic models. Their compelling conclusion is that the APULVZ is a magma

Cilia Discern Left from Right

Dominic P. Norris and Daniel T. Grimes

Science **338** (6104), 206-207.
DOI: 10.1126/science.1230401