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Development of the asymmetric human

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Symmetry across the midline is present in many animals, together with the left/right asymmetry of several organs, such as the heart in vertebrates. The development of such asymmetries during embryonic development requires first the specification of the midline and then specification of left/right. One model proposes the transfer of molecular asymmetry to the multicellular level. Nodal expression on the left side in mammals and chicks is a key event, and is due to the release of calcium on the left possibly involving an ion pump and the Notch pathway

Vertebrates, humans included, are bilaterally symmetrical about the midline of the body for many structures, such as eyes, ears and limbs. While the body is outwardly symmetrical, most internal organs are asymmetrical with respect to the left and right sides.[1] For example in mammals, including humans, the heart is on the left side, the right lung has more lobes than the left, the stomach and spleen lie to the left, and the liver has a single right lobe. This handedness is remarkably consistent, but there are rare normal individuals, only about one in 10,000, who have the condition *situs inversus,* which results in complete mirror-image reversal of the asymmetry. However, such reversal can also occur with the condition known as Kartagener's syndrome, a recessive genetic disorder, which also affects the movement of cilia in the respiratory organs such as the lungs. There is also evidence that many congenital abnormalities of the heart are related to errors in the process of specifying left/right during heart development.

Specification of left and right during embryonic development is different from specifying the other axes of the embryo such as antero-posterior, and dorso-ventral. Embryos develop along axes not all that different from a simple three-dimensional Cartesian system. One could not tell someone by phone in a room on Mars which side of a body is left and which right, unless they could look

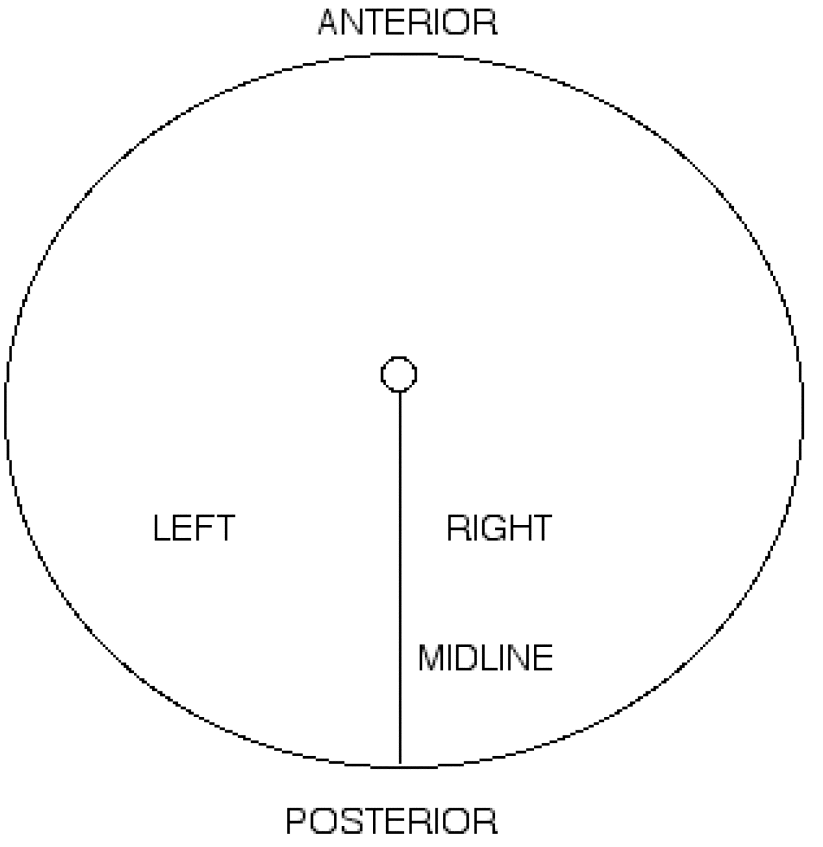

Figure 1. In mammals and the chick there is a midline that defines bilateral symmetry. The arrow points anteriorly and is where the organizer is at the node.

outside or have insight to beta-decay which has a left–right asymmetry. Left and right only have meaning once the antero-posterior (head to foot) and dorso-ventral (back to front) axes have been defined. If one of these axes is reversed, then so too will the left–right axis be reversed. It is for this reason that when you look in a mirror that your left hand looks like a right hand – your dorso-ventral axis has been reversed. Thus a problem in development is to understand how left and right are established after the other axes have been specified.

A crucial step in animal evolution was the emergence of the bilateral body plan,[2] which requires a midline organizer (Figure 1). The way this is specified varies considerably. In the fly Drosophila, the midline is initiated in the mother when the egg develops, and after fertilization there is a dorso-ventral gradient in one protein in the nuclei that has it high point ventrally, and another gradient with its

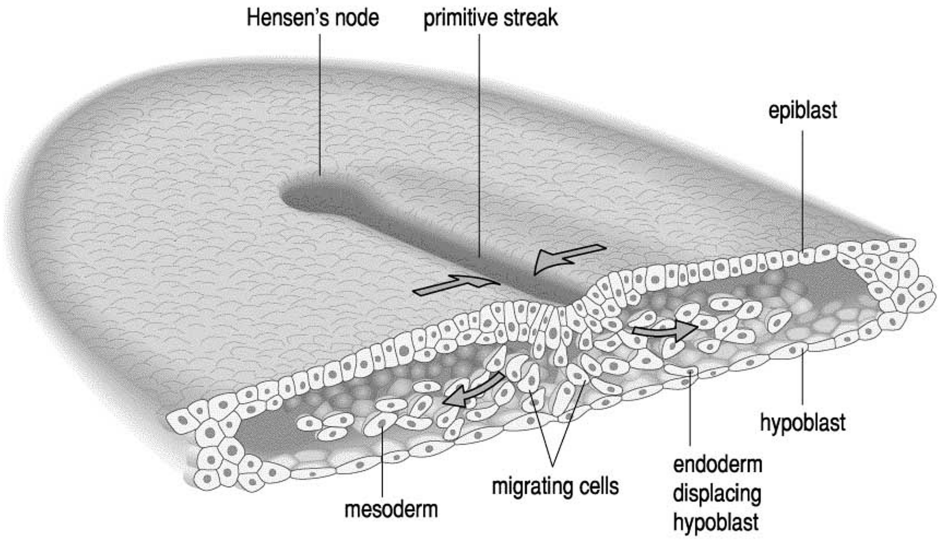

Figure 2. The primitive steak of the chick showing the position of the node anteriorly.[1]

high point dorsally. In the nematode worm, left–right asymmetry is specified very early, at the six-cell stage, when there is a slight left–right difference of the arrangement of the cells along the future body axis – the left–sided cells are slightly anterior to those on the right. If this arrangement is manipulated so that the right side is ahead, then asymmetry is reversed. In higher vertebrates, like the chick and mouse, as well as humans, the dorsal organizer, at the future posterior end, causes the initiation and elongation of a streak, along the midline in both anterior and posterior directions. The relationship of the initiation site to the early cleavages is a bit controversial. The maintenance of the straightness of the line may be due to strong lateral inhibition by the organizer. At the tip of the streak is the node, the site of the organizer (Figure 2). The streak also defines the dorso-ventral axis.

Once bilateral symmetry is established then there must be a mechanism for specifying the difference between the left and right sides. It is the sequential action of genes, and the proteins they code for, that leads in the embryo to differences in the development of organs with respect to the midline. But how is left as distinct from right side specified?

Some time ago Nigel Brown and I[3] proposed that this L/R asymmetry must be due to some molecular asymmetry that is transferred to a global asymmetry. Our model was based on an F-shaped molecule that was oriented with respect to both the antero-posterior and dorso-ventral axes (Figure 3). It is at once clear that there are differences between the left and right sides; for example if the arms of the

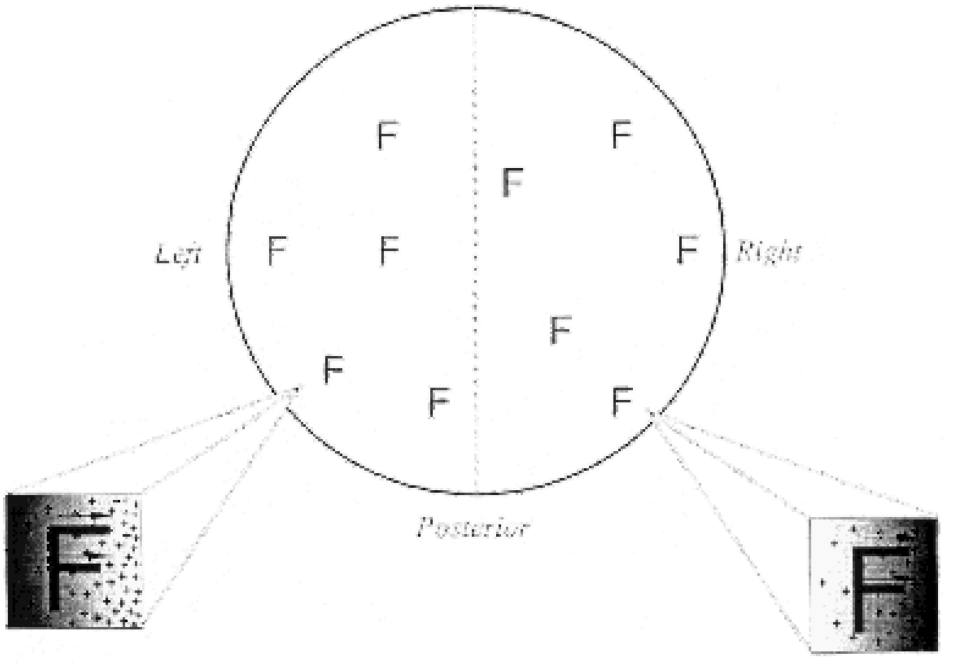

Figure 3. A mechanism by which an asymmetrical F-shaped molecule could lead to left–right asymmetry at the multicellular level. The molecule needs to be oriented with respect to the dorso-ventral and antero-posterior axes. Then, if the arms of the F pump molecules to their ends then there will be a clear L/R difference across the midline.

F transported some molecule to their ends then on the left side movement would be towards the midline, but on the right it would be away from the midline. It is of interest that cilia are asymmetrical and can be characterized by an F-shaped molecule (Figure 4), so if the cilia were oriented with respect to the antero-posterior and dorso-ventral axes the left and right sides would be different and the flow caused by the motion of the cilia would be from left to right. If one looks at the cilia on the ventral surface of a tadpole's skin this is what one would see.

The next steps would be to activate specific genes on one side but not on the other, the stabilization of such differences, and then the translation of these differences into asymmetric organ development. A key set of molecules in the development of asymmetry in vertebrates is related to the protein Nodal, which is a member of the transforming growth factor-beta superfamily, whose signals are widely used in the development of the embryo. In the mouse and chick, both very good models for human development, the gene *Nodal* is expressed both in

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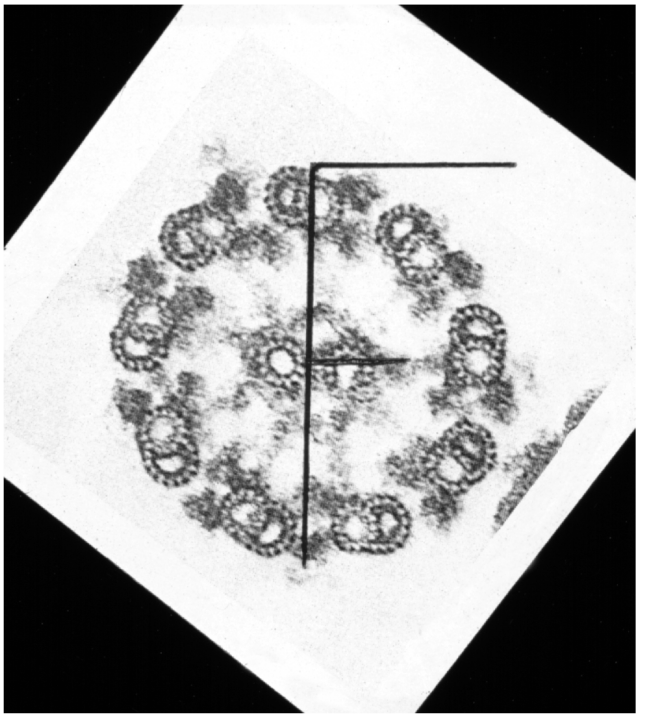

Figure 4. Cross-section of a cilium to show its asymmetry; an F-shaped molecule reflects this asymmetry.

the central region, Hensen's node, as well as laterally in the lateral plate mesoderm (Figure 5). Expression in the lateral region is dependent on expression in the node. Mutations in *Nodal* result in loss of normal asymmetry.[4] In conjoined twins, the twin on the right side often has inverted symmetry because of inhibition due to the left twin.

How is it that *Nodal* is expressed only on the left side. *Nodal* in the mouse embryo is initially expressed transiently on the left of the node at the anterior end of the primitive streak, and this expression is necessary for the later expression of *Nodal* in the left lateral plate mesoderm. The key expression on the left of the node involves the Notch signalling pathway.[5] Notch is a receptor on the cell membrane and its activation can lead to a variety of cellular responses. Its ligand, Delta, when knocked out in mice results in a lack of L/R asymmetry. Using the chick embryo as a model, where Nodal is clearly expressed on the left of Hensen's node, it was shown that an increase in calcium on the left side led to the activation of the Notch pathway and was followed by a *Nodal* expression on that side. *Nodal* maintains its own expression and activates a series of genes on the left side that

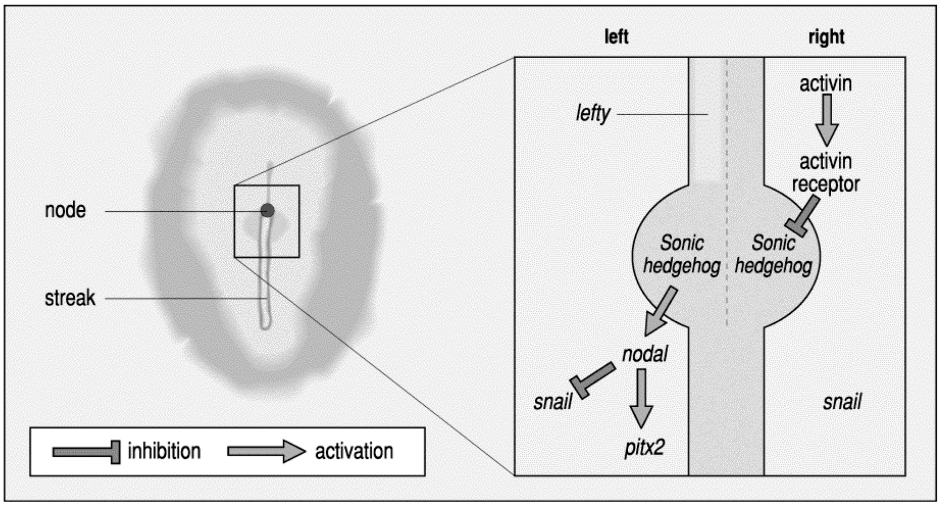

Figure 5. A simplified view of some of the genes involved in L/R asymmetry in the chick. Inhibition of sonic hedgehog on the right side leads to nodal expression on the left side.[1]

in turn lead to organ asymmetry. But the details of the mechanism by which, for example, the heart moves to the left remains unclear.

But what causes the increase in calcium on the left side? In the chick embryo the proposed mechanism is based on left/right differences in H^+/K^+-ATPase activity. H^+/K^+-ATPase activity across Hensen's node creates a gradient in membrane potentials, which in turn is likely to cause a differential ion flux across the L/R axis, so that calcium accumulates on the left side.[6] In the mouse, a rather different mechanism for accumulation of calcium on the left side has been proposed.[7] In the mouse node, and so presumably also in humans, there are two populations of cilia. One motile type of cilia moves fluid from the right side to the left side and this bends the non-motile sensory cilia on the left side, this in turn results in the release of calcium and *nodal* activation. Artificial reversal of flow reverses left–right asymmetry.

The human brain has many asymmetrical features and how these are specified is poorly understood, even if the mechanism that is involved in the development of body L/R asymmetry also controls the development of asymmetry in the brain. A nice genetic model for left- and right-handedness has been put forward by McManus.[8] He assumes that there are just two genes controlling handedness, a *dextral* gene, D, and a *chance* gene C. People with two copies of the D gene, who are thus DD, will always be right handed; those who are CC will be left or right 50% of the time, and CD individuals have a 25% chance of being left-handed.

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References

1. L. Wolpert, R. Beddington, T. Jesell, P. Lawrence, E. Meyerowitz and J. Smith (2002) *Principles of Development* (Oxford University Press).
2. H. Meinhardt (2004) Different strategies for midline formation in bilateralians. *Nature Reviews Neuro*, **5**, 502–509.
3. N. Brown and L. Wolpert (1990) The development of handedness in left/right asymmetry *Development*, **109**, 1–14.
4. A. Raya and J. C. Izpisua Belomonte (2004) Sequential transfer of left–right information during vertebrate embryo development. *Curr. Opin. Genetics & Development*, **14**, 575–581.
5. A. Raya, Y. Kawakami, C. Rodrıguez-Esteban, M. Ibanes, D. Rasskin-Gutman, J. Rodrıguez-Leo, D. Buscher, J.A. Feijo and J.C. Izpisua Belmonte (2004) Notch activity acts as a sensor for extracellular calcium during vertebrate left–right determination. *Nature*, **427**, 121–128.
6. Levin, M., Thorlin, T., Robinson, K.R., Nogi, T., and Mercola, M. (2002) Asymmetries in H^+/K^+-ATPase and cell membrane potentials comprise a very early step in left-right patterning. *Cell*, **111**, 77–89.
7. J. McGrath, S. Somlo, S. Makova, X. Tian and M. Brueck, M. (2003) Two populations of node monocilia initiate left-right asymmetry in the mouse. *Cell*, **114**, 61–73.
8. C. McManus (2002) *Right Hand, Left Hand* (Weidenfeld).

About the Author

Lewis Wolpert is Emeritus Professor of Biology as Applied to Medicine at University College London. He was originally trained as a civil engineer in South Africa but changed to research in cell and developmental biology in 1955. His main contributions are related to morphogenesis and the concept of positional information. He has been Chairman of the Committee for the Public Understanding of Science. His books include *Malignant Sadness – The Anatomy of Depression* (Faber, 1999) and *The Unnatural Nature of Science* (Faber, 1992). He also writes a column for *The Independent* newspaper.