

Developmental biology

Asymmetrical threat averted

Eran Hornstein and Clifford J. Tabin

The somites are embryonic elements that give rise to the muscles, skeleton and some skin layers of the trunk. They form in a symmetrical fashion, but to do so they must be shielded from asymmetrical cues.

The human body looks deceptively symmetrical from the outside. In contrast to this external symmetry, our internal organs are in an asymmetrical, yet reproducible, arrangement. The heart is on the left and the lung next to it is smaller than that on the right. Within the abdominal cavity, the intestines form a clockwise loop; the stomach, pancreas and spleen are on the left, whereas the liver and gall bladder are on the right. But our body plan does not start out this way, and some body parts — the somites, for instance — must remain bilaterally symmetrical. A striking series of findings by Kawakami *et al.*¹ and Vermot and Pourquié², published on pages 165 and 215 of this issue, along with a complementary manuscript in *Science*³, teach us how somite formation proceeds in a bilaterally synchronized fashion (Fig. 1). Furthermore, Kawakami *et al.* and Tanaka *et al.*⁴, also writing in this issue (page 172), shed new light on how asymmetry, which is necessary for the physiological function of many organs, is first established in the embryo (Box 1, overleaf).

The left–right differences seen in many of our internal organs are rooted in a cascade of molecular asymmetries that is established during development (Box 1). Discoveries over the past decade have provided an increasingly detailed picture of how such left–right asymmetry is imposed on the developing embryo⁵. However, regulation of the alternative — bilaterally symmetrical morphogenesis — has not been given serious attention.

As the embryo begins symmetrically, it is

perhaps natural that researchers have intuitively viewed symmetry as a default state. For instance, this view has been applied to the somites, which give rise to tissues of the body wall, such as the musculature, skeleton and some skin layers. Somites are generated bilaterally in symmetrical pairs, with one somite of each pair on each side of the developing spinal cord. This process is driven by bilaterally symmetrical waves of gene expression⁶. The first pair of somites emerges at the anterior part of the embryo (next to the head), and more caudal pairs of somites form successively in an anterior–posterior sequence.

Kawakami *et al.*¹, Vermot and Pourquié² and Vermot *et al.*³ now show that somite formation can proceed in a bilaterally synchronized fashion only because somites are actively masked from information that would otherwise cause them to develop asymmetrically. Experiments in mammals, birds and fish all yield clues that somites are masked from left–right cues, and so are held in symmetrical register, by a signal that depends on retinoic acid (RA). When RA activity is experimentally attenuated, bilateral segmentation is taken out of phase.

This symmetry-generating mechanism is necessary because, to develop properly, somites need to be refractory to left–right signals that are present in their physical territory. In principle, somite cells could simply ‘ignore’ such cues by not expressing the receptive components needed to interpret them (if, for instance, they did not produce receptors for the relevant growth factors).

But what profoundly complicates things is that the very same left–right signalling cues are used to pattern somites along the anterior–posterior axis.

This problem of common signals being put to conflicting use finds its origin in a key principle that has emerged in modern molecular embryology. A surprisingly small number of molecular signals — such as Wnt, Notch, Hedgehogs, bone morphogenetic proteins and fibroblast growth factors (FGFs) — are repeatedly employed in different developmental settings, often in conjunction with distinct cofactors, to orchestrate differentiation and patterning. As in the composition of a musical piece, biological motifs are used over and over again, with changes of context and emphasis to generate new interpretations.

The anterior–posterior patterning of somites, for instance, is specified by the combined action of the Notch, Wnt, FGF8 and RA signalling pathways⁶. At the same time, the Notch, FGF8 and RA pathways are also instrumental in left–right determination⁵. This conflict makes it necessary to block the effect of side-specific signalling, to hold somitogenesis in synchronized and symmetrical register. That need is met, the new papers show^{1–3}, by an RA-dependent mechanism. More broadly, these new findings suggest that conflicting developmental pathways in general may be intermingled in a harmonious way through buffering mechanisms.

We have a lot to learn yet about such buffering mechanisms. In this particular case, it is still unclear exactly how RA mediates the masking of left–right signals. Might this molecule be differentially spread in the left and right somitic fields? On the basis of the expression patterns of messenger RNAs encoding RA-metabolizing enzymes, Kawakami *et al.*¹ suggest that the answer is no. However, a relevant enzyme might still be differentially regulated at a post-transcriptional level.

Another question concerns the relevant molecular targets of RA. RA is known to antagonize FGF8, which is generated at the posterior end of the embryo during somitogenesis. This antagonism is crucial for segmentation to proceed⁶, but it would be interesting to investigate whether the FGF8–RA interface also serves a role in buffering left–right signalling. An alternative protein that might buffer the system is Lefty-1, which is an important inhibitor of side-biasing information flow, although it acts at a stage downstream from the factors that impinge directly on the somites. Consistent with this possibility, the production of Lefty-1 is induced by RA and occurs in the midline of the embryo.

The new studies^{1–3} might also have clinical relevance. RA is produced from vitamin A in the body, so a maternal shortage of

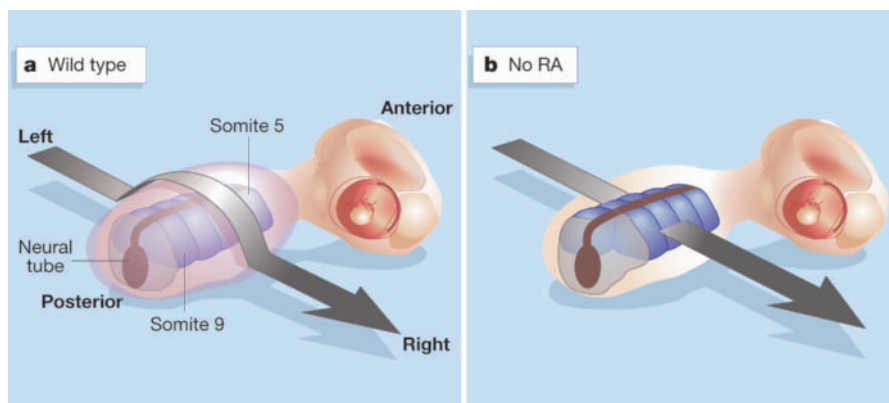


Figure 1 Maintaining symmetrical somitogenesis. A vertebrate embryo is shown from the back. a, The new papers^{1–3} show that, in wild-type embryos, a protective effect of retinoic acid (RA; pink field) masks the flow of left–right information (black arrow) to enable the symmetrical bilateral formation of new somites (blue). b, Blocking RA production exposes somites to left–right signals and takes their generation out of synchrony.

Box 1 Generating asymmetry

Over the past decade, much has been learnt about the genetic pathways that direct left–right asymmetry. A major remaining controversy has been the mechanism by which symmetry is first broken, in a reproducible orientation, in the early embryo.

In mammals, the initial symmetry-breaking event is believed to take place in the node — a small, transient pit at the anterior end of the primitive streak, near the middle of the early embryo. Thus, in mice, the first observable left–right difference is the directional rotation of microcilia (hair-like structures), which sets in motion a leftward flow of extracellular fluid across the node.

Two alternative mechanisms have been proposed for how fluid flow is sensed by the embryo: the unidirectional transport of a morphogen protein, and the

asymmetric deformation of mechanosensory cilia (the ‘two-cilia’ hypothesis; see ref. 4 and references therein). Tanaka *et al.*⁴ provide the first experimental support for the former model, showing that Sonic hedgehog (Shh) protein and retinoic acid are encapsulated, in a process that depends on fibroblast growth factor, into membrane-wrapped ‘nodal vesicular parcels’ (NVPs), which are asymmetrically transported by the flow across the node.

The biological significance of Shh transport remains unclear, however, as previous studies⁸ failed to detect any asymmetric readout of the Hedgehog pathway at the mouse node. Further, in the face of normal nodal hydrodynamics, mice with dysfunctional mechanosensory cilia exhibit defects in handedness⁹ that are consistent with the ‘two-cilia’ model, but are left unexplained in

the context of the NVP hypothesis. The discovery of the NVPs is intriguing, yet in its wake there are enough contradictions and open questions to stress the need for a unified interpretation that can explain all the existing data, and for an experimental setting that can definitively exclude one of those models.

Whichever way their action is interpreted, nodal cilia do seem to be at the root of mammalian symmetry breaking. In amphibians and birds, however, asymmetries are detected before the node forms, in some proteins of the extracellular matrix and in the activities of H⁺/K⁺-ATPase channels and Notch proteins. This raises the possibility that the cilia-induced flow might be a mechanism unique to mammals.

However, Kawakami *et al.*¹, and Essner *et al.*⁹, show that ciliary movement and leftward flow are conserved early steps in

establishing left–right asymmetry, and verify that the activity of the microciliary motor protein (encoded by the *lrd* gene) is required for microcilia activity in zebrafish, as in mice. Yet Kawakami *et al.* also show that, in zebrafish, H⁺/K⁺-ATPase and Notch act upstream of *lrd* and of the flow generated by microcilia. They conclude that the cilia may act to transduce the early left–right pattern that is initially established by H⁺/K⁺-ATPase and Notch.

It remains to be determined how the H⁺/K⁺-ATPase and Notch signalling systems produce a consistent left–right orientation, and how these earlier signals are connected to the latter effects of extracellular fluid flow at the node. Another question is whether these initiating steps are conserved in all vertebrates, or whether they have been lost in mammals, with the nodal flow being the true symmetry-breaking event. **E.H. & C.J.T.**

vitamin A during pregnancy might be associated with increased rates of fetal skeletal defects, such as hemivertebrae and scoliosis. The World Health Organization has found vitamin-A deficiency to be common in south Asia and Africa, suggesting that millions of pregnancies a year are carried by women with a vitamin-A shortage⁷. This astonishing number of pregnancies at potential risk might promote epidemiological studies of this theoretical correlation. ■

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Planetary science

Magnetic impact craters

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Aerial surveys of the Vredefort impact crater in South Africa suggest that it is only weakly magnetic. The rocks themselves tell a different story, but does this apply to giant impact basins on Mars?

Evidence about the history of Mars can be gleaned from magnetic surveys — hence the importance of data sent back by the Mars Global Surveyor in the 1990s¹. The magnetometer on the satellite measured much stronger magnetic fields over some parts of the southern highlands of the planet than fields at similar altitudes over Earth. But the fields are notably weak over the giant impact basins Hellas and Argyre (Fig. 1), suggesting that the dynamo in the martian core was inactive during the era of major

meteorite impacts 3.5–4 billion years ago^{2–4}. This conclusion has had a considerable influence on ideas about how Mars cooled, and when its mantle and inner and outer cores differentiated.

The hypothesis of demagnetized impact basins and all it implies about Mars’ evolution is called into question by Carporzen *et al.*⁵ on page 198 of this issue. They measure unusually strong magnetizations of bedrock samples in the giant Vredefort impact crater of South Africa, yet aerial measurements

of magnetic fields over the crater are lower than over surrounding areas. This paradox is explained by variations in the directions of sample magnetization vectors over distances of 10 cm or less. The strong, but spatially incoherent, magnetic signal of the bedrock is essentially randomized when viewed from larger distances. Meteorite craters can then seem to be magnetic or non-magnetic, depending on how close the magnetometer is to the source. Viewed from satellite altitudes of 100–400 km, martian impact basins would appear magnetically featureless if the magnetic vectors of their source rocks vary in direction over distances of a few kilometres or less.

The Vredefort crater is 2 billion years old and was originally 300 km in diameter. Vredefort is Earth’s oldest and largest impact structure, and Carporzen *et al.*⁵ argue that, as such, it is our best analogue for the giant martian impact basins. Moreover, other terrestrial impact structures, the Charlevoix and Slate Islands impact craters in Canada among them, also have strong but spatially dispersed sample magnetizations and low aeromagnetic signatures.

But what causes this unusual behaviour? Rocks typically become magnetized when they are cooled from a melt or other high-temperature conditions. This thermal remanent magnetization, like virtually all other mechanisms of magnetization, forms parallel to the magnetic field that acts during cooling of the magnetic minerals in the rock⁶. This parallelism is central to the success of