Is your heart on the "right" side? It's a matter of a "rightward" stroke of cilia at the right developmental moment

All animals have distinct head-tail (anterior–posterior), and top–bottom (dorsal–ventral) structures. When examined externally, many animals appear bilaterally symmetric but the symmetry breaks down if one looks inside. The vertebrates are perhaps the best example of such lateral asymmetry called *situs*. The heart is placed on the left, the liver on the right etc., and though the limbs are mirror images of one another, our brain can easily distinguish between the left and right. How does this handedness or *situs* develop? Until recently this was, perhaps the single most fundamental enigma in developmental biology and inspired several hypotheses (reviewed in Brown and Wolpert 1990). A string of recently published research articles provide concrete evidence to suggest a novel way of generating such axial asymmetry (Nonaka *et al* 1998; Marszalek *et al* 1999; Takeda *et al* 1999). These studies show that a leftward flow of extra embryonic fluid is propelled by spinning cilia at the node of a gastrulating embryo, and suggested that this is essential to generate the handedness or *situs* in mice. The following paragraphs elaborate the complexity of this problem and the significance of recent results.

How to make the "left" different from the "right"?

The left–right (L/R) axis is likely to be defined once the anterior–posterior (A/P) and dorso-ventral (D/V) axes are fixed. In one of the classic experiments, Speamann and Falkenberg (1919) could produce twin headed newts with mirror image duplication of internal asymmetry by tying a hair between the two blastomeric spheres of a salamander embryo. This suggested that the asymmetry is set very early during development, and, communication between the two lateral sides is essential. It is observed that vertebrate embryos turn to the right side at an early stage of gastrulation (see figure 1). This is the earliest indication of handed asymmetry in physical structure during development. This turning phenomenon seems to have a bearing on the leftward looping of the heart and the placement of other internal organs. Several other observations indicate that an asymmetry in chemical composition of the embryonic cells sets in even before the turning. In mouse embryos such an asymmetry is first observed at 7·5 days after fertilization, when the cells on the left side start producing more *nodal* mRNA than their right side counterparts (Collignon *et al* 1996). This is seen in cells of developing mesoderm (lpm; figure 1), which forms muscles in adult, and in the left side of a region called the node (figure 1). A similar left specific expression of some *nodal-related* genes are observed in frog (*xnrI*; Sampath *et al* 1997) and chicken (*cnrI*) embryos at equivalent stages (Levine 1997). The nodal and other nodal related proteins are similar to the transforming growth factor TGFβ and they are perhaps secreted by the cells. Once the nodal gene expression gets localized in the left side cells, a host of other proteins (Lefty, Ptx2, etc.,) are produced in these cells and thus a left specific chemical identity develops. The Jury is, however, still out on, how the leftward expression of nodal etc., are initiated.

A clue came from the investigation of a mouse mutant, called *inversus viscerum* (*iv*), which suggested that the left specific expression of *nodal* and the rightward turning of the embryo are linked. *iv/iv* embryos show laterally-symmetric *nodal* expression and have randomized L/R-asymmetry in the viscerum (Collignon *et al* 1996). The mutation in *iv/iv* animals was found to be in a gene, called "*left-right-dynein*" (*lrd*), which is expressed at a higher level in the nodal cells (figure 1) at day 6·5

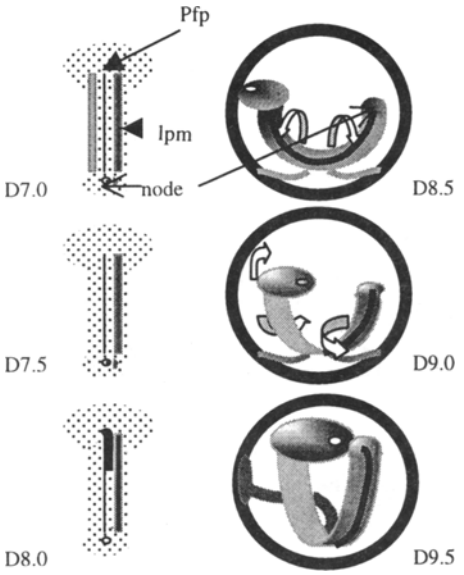

Figure 1. A time-lapse description of mouse embryo at the gastrula stages. The right side panel indicates dorsal view of a flattened mouse embryo with the anterior side up. Solid gray shading indicates nodal gene expression in the first two frames while solid black shades indicates lefty gene expression in the last frame. The left side panel depicts lateral view of the embryos inside the yolk sac marked by solid black circle. At day 8·5 after fertilization a typical mouse embryo looks like a tadpole floating inside a yolk sac and anchored to the outer membrane at the mid-ventral region. The embryo starts turning right ward, as shown by curved arrows, with respect to the AP axis and inverts completely by day 9·5. The primitive streak (Pfp), lateral plate mesoderm (lpm) and node are indicated by arrows.

after fertilization (Supp *et al* 1997). The Lrd protein, as its name suggests, is similar to dynein, and it may function in maintaining cilia movement. Interestingly, analysis of a human disease also suggested the possibility of a dynein being involved in the left–right decision making process. Humans with Kartagener's syndrome often have their heart on the "right" side and also suffer from chronic sinusitis. Further, the dynein arm of sperm-ciliary-axonemes are missing in males with Kartagener's syndrome, resulting a loss in sperm motility (reviewed in Afzelius 1995).

How do motor proteins, apparently involved in moving cilium, control L/R developmental decisions?

To answer this question one has to first show that cilia are indeed involved in this process. Previous studies had established the presence of cilia on the nodal cells, but a direct evidence of their role in *situs* was lacking. The issue is clinched by the discovery that mice knockouts of kinesin-like-proteins, KIF3B and KIF3A, affects ciliogenesis at the node and cause *situs inversus* (Nonaka *et al* 1998; Marszalek *et al* 1999; Takeda *et al* 1999). These studies showed that the KIF3A$^{-/-}$ and KIF3B$^{-/-}$ embryos display laterally symmetric expression of some otherwise left-specific genes and they failed to turn toward right. This focuses the issue firmly on cilia, because proteins similar to KIF3A and B are involved in cilia and flagella movement in sea urchin and in the blue-green algae *Chlamydomonas* respectively. Cells at the node of a 7·5 day old mouse embryo do have cilia like structures, and these are drastically shortened in both KIF3A$^{-/-}$ and KIF3B$^{-/-}$ embryos. Nonaka *et al* (1998) made a significant further contribution by showing that the nodal cilia in mice are indeed motile. Fluorescent beads injected at the node region of developing mouse embryos moved leftward indicating a flow of the fluid around the embryo inside the yolk sac. This leftward flow, they suggested, is caused by a counter clockwise movement of the nodal cilia. As expected, both the KIF3A$^{-/-}$ and KIF3B$^{-/-}$ embryos were deficient in cilia movement and in "nodal" flow. The authors explained that the leftward flow

created by the nodal cilia must help to concentrate certain factors (morphogens) which may induce and maintain the left specific gene expression in the embryo. It is also likely that the net leftward flow, caused by counter-clockwise turning of cilia at the node, will produce a rightward reaction on the embryo itself and result a rightward turning (figure 1).

If these conclusions are correct then just turning the nodal cilia clockwise should result a complete visceral inversion. That might be the case in another mouse mutant, called *inv*, where the asymmetry in nodal expression is reversed in homozygous mutant embryos. But one may still ask, why do the nodal cilia normally turn counterclockwise?

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Ploidy influences gene expression in yeast

Genomes are usually regarded as static, changing only on the leisurely time-scale of evolution. This assumption clearly overlooks the changes in ploidy that cells in an organism undergo during various stages of growth and development. A mitotic cell doubles its ploidy during DNA synthesis and restores it subsequently at cell division. Polyploid cell types such as megakaryocytes (16n to 64n) or hepatocytes (2n to 8n) are commonly found during normal differentiation. Tumour cells have aberrant cell-cycle controls leading to an altered ploidy status. Further, deviation from the common theme of a haploid/diploid genomic constitution is widespread in the plant kingdom.

Do changes in the ploidy of a cell influence gene expression? Halving or doubling the total size of the genome would leave *relative* gene dosages unaffected; so can one expect patterns and relative levels of gene expression to remain identical? In the special case of *reduced* ploidy the answer is