Left, right ... which way to turn?

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When we look into a mirror, it is obvious that we have two eyes, two arms and two legs, one on the left and one on the right. On the inside, there is only a single heart, stomach, liver and spleen, but Nature seems to have developed an intricate method for placing these unpaired organs in a highly ordered pattern along the left–right (LR) axis of all vertebrates. The heart points to the left; the liver resides on the right, and the stomach and spleen are on the left (Fig. 1*a*). On rare occasions, people are born with complete mirror-image reversal of this intricate asymmetry of the visceral organs—a condition known as *situs inversus* (Fig. 1*b*). Surprisingly, this condition is often not associated with any pathological abnormalities and may be detected only incidentally on X-rays. A more unfortunate condition is when the heart-, lung- or gut-sidedness is random—a condition known as *situs ambiguus* or heterotaxy (*hetero* meaning 'other' and *taxy* meaning 'arrangement') syndrome (Fig. 1*c*). This condition is almost always associated with disease of the visceral organs. How are our organs normally patterned with such precise LR asymmetry, and what goes awry when they are mispatterned?

> "The most frequent of the internal misplacements is that in which the heart is placed in a similar position on the right side of the chest to that which it should occupy on the left...When this occurs, the viscera of the body generally are also most usually transposed; but such is not always the case..."[1]
>
> —Thomas Peacock, 1858.

Two striking new discoveries shed light on the molecular events regulating the establishment of the asymmetric LR body plan. In this issue of *Nature Genetics*, Marinella Gebbia and colleagues identify *ZIC3*, encoding a zinc-finger transcription factor, as the gene mutated in a family with X-linked inheritance of *situs* abnormalities[1]. This is the first gene associated with LR patterning defects in humans, and the authors describe its mutation in both familial and sporadic cases[2]. A spontaneously occurring mouse model of heterotaxy syndrome, *inversus viscerum* (*iv/iv*), has been instrumental in the understanding of molecular pathways controlling LR asymmetry[3]. In a recent issue of *Nature*, Dorothy Supp and colleagues attributed the *iv* phenotype to a mutation in an axonemal dynein heavy chain, a microtubule-associated cytosolic motor[4]. Together, these are the first demonstrations of single-gene defects that lead to naturally occurring LR patterning abnormalities in human and mouse. How might mutations in these two seemingly unrelated proteins result in heterotaxy syndrome?

A cascade of signalling molecules regulating the establishment of embryonic LR asymmetry is indicated by recent studies of chick embryonic development (Fig. 2*a*). Before the formation of organs in the developing embryo, asymmetric expression of *Sonic hedgehog* (*Shh*) leads to left lateral mesoderm expression of *nodal*, a member of the transforming growth factor–β (TGF-β) family[5]. Left-sided expression of *nodal* is responsible for rightward looping of the midline heart tube, the first overt sign of embryonic LR asymmetry[6,7]. Expression of *Shh* and *nodal* is suppressed in the right lateral mesoderm by an activin-receptor mediated pathway[5]. Conversely, the snail-related (cSnR-1) zinc-finger transcription factor is expressed in the right lateral mesoderm and is repressed by *Shh* on the left[8]. In mice, unlike chicks, *Shh* is not expressed asymmetrically, although *nodal* and another TGF-β member, *lefty*, retain their left-sided expression; this asymmetric expression pattern is reversed in *situs inversus* mice (*inv/inv*) and randomized or absent in *iv/iv* mice[9–12]. The intricacies of how asymmetry of gene expression is established and maintained is a mystery, but it is suspected that signals emanating from the midline notochord may play a role[13,14].

Among the many hedgehog genes in vertebrates, *Shh* is expressed in the vertebrate notochord, where it probably plays a role in embryonic patterning[15]. Unlike vertebrates, fruit flies have only one hedgehog protein, Hh, and much has been learned about Hh signalling in flies, in which it imparts the spatial informa-

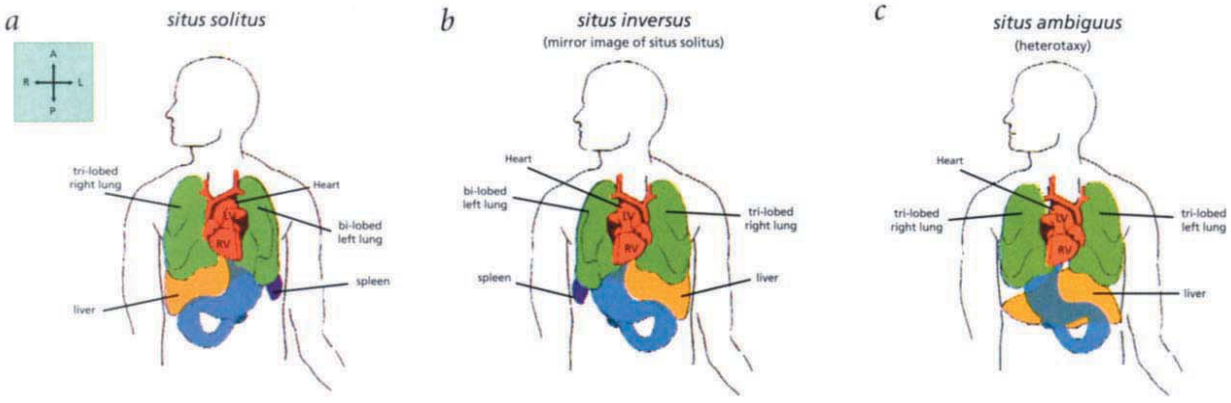

Fig. 1 Abnormalities of LR asymmetry in humans. In response to a cascade of signalling molecules, the normal asymmetric pattern of visceral organs, *situs solitus*, is established (***a***). The heart points to the left (LV, left ventricle; RV, right ventricle) and the aortic arch curves to the left. The stomach and spleen lie on the left, while the liver is on the right. The right lung has three lobes, whereas there are two lobes on the left lung. The gut coils itself inside the abdomen in a counterclockwise rotation. In *situs inversus*, there is a complete mirror image reversal of asymmetrical organ position (***b***). This can occur in a coordinated fashion and presumably arises from complete reversal of the asymmetric cascade of embryonic signalling that establishes LR asymmetry. Heterotaxy syndrome or *situs ambiguus* is characterized by randomization of LR placement of visceral organs and is probably due to the absence of coordinated LR signalling (***c***). The lungs, heart, liver, spleen and stomach are oriented randomly with respect to the LR axis and with respect to one another.

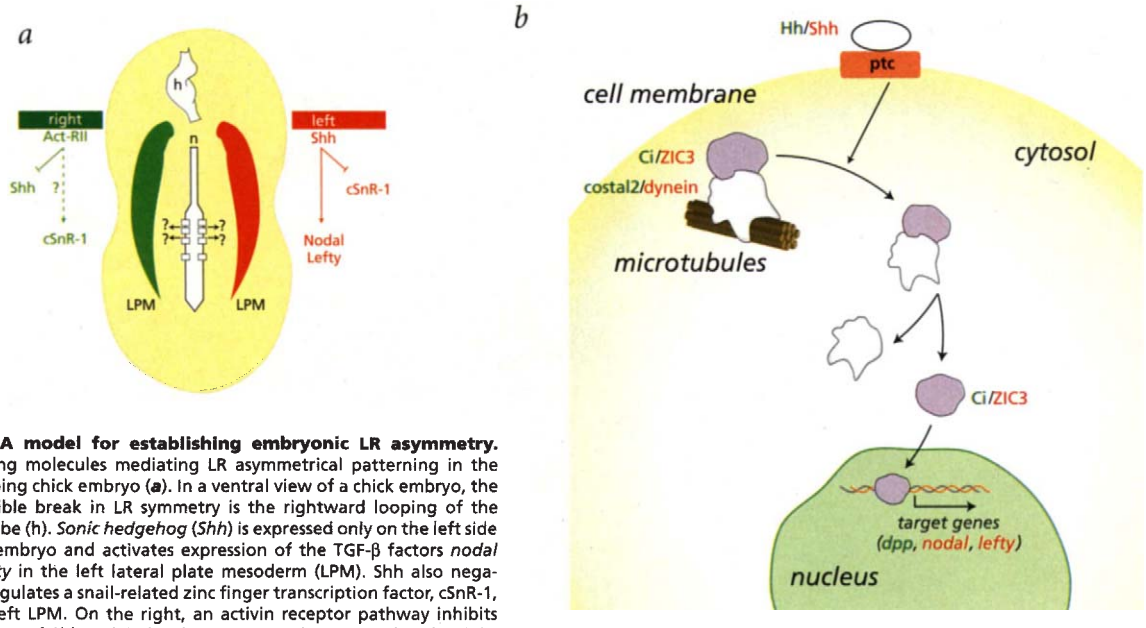

Fig. 2 A model for establishing embryonic LR asymmetry. Signalling molecules mediating LR asymmetrical patterning in the developing chick embryo (***a***). In a ventral view of a chick embryo, the first visible break in LR symmetry is the rightward looping of the heart tube (h). *Sonic hedgehog* (*Shh*) is expressed only on the left side of the embryo and activates expression of the TGF-β factors *nodal* and *lefty* in the left lateral plate mesoderm (LPM). Shh also negatively regulates a snail-related zinc finger transcription factor, cSnR-1, in the left LPM. On the right, an activin receptor pathway inhibits expression of *Shh* and, in its absence, *cSnR1* is expressed in the right LPM. The notochord (n) and somites (open squares) are seen in the midline. As yet unidentified signals from the midline play a role in determining LR asymmetry. Shh, Zic3 and dynein are expressed in the midline of the developing embryo. (***b***) A hypothetical model for Zic3 and the role of dynein in LR asymmetry. In *Drosophila*, the microtubule-associated motor Costal2 interacts with a Gli-family transcription factor, cubitus interruptus (Ci) and sequesters it in the cytosol. A signalling pathway initiated by hedgehog (Hh) releases the Costal2-Ci complex from the microtubules and allows Ci to enter the nucleus where it mediates Hh signalling by activating target genes such as *dpp*, a member of the TGF-β family. An analogous pathway may exist in vertebrates where a microtubule-associated anonemal dynein sequesters the vertebrate Gli-like protein Zic3 by forming a complex with it on the microtubule. A sonic-hedgehog (Shh) initiated pathway may release the dynein-Zic3 complex, allowing Zic3 to enter the nucleus where it activates target genes such as those encoding the TGF-β factors nodal and lefty. In this model, Zic3 and dynein would cooperate in mediating the sonic-hedgehog signalling pathway to establish LR asymmetry; this would be consistent with mutations in both proteins causing similar phenotypes.

tion required for the development of segmental polarity[16]. Signal transduction of Hh is mediated by activators and repressors of Hh target gene expression. One such mediator, *cubitus interruptus* (*ci*), encodes a member of the Gli family of transcription factors; Ci is necessary and sufficient to mediate the Hh signal and is regulated in a fashion dependent upon a gradient of midline Hh expression[17,18]. A downstream target of *Ci* is *decapentaplegic* (*dpp*), a gene encoding a TGF-β factor that is also involved in establishing segment polarity in flies[19]. Odd-paired (Opa), which shares homology with vertebrate Zic proteins, is also an Hh mediator and establishes segmental identity in flies by activating transcription of *wingless* (homologous to the *Wnt* family in vertebrates)[20]. The finding that Gli-type *ZIC3* is mutated in human heterotaxy syndrome reveals clues to how such midline signals may control vertebrate LR asymmetry. As the mouse homologue Zic3 is expressed in the notochord before initiation of the asymmetric LR signal cascade[21], one could speculate that, in vertebrates, Shh may modulate the activity of Zic3—which, in turn, positively or negatively regulates expression of the TGF-β factor *nodal* (Fig. 2*a*,*b*). This would be analogous to the *hh-ci-dpp* pathway described in flies. Unlike flies, however, higher organisms have multiple Gli proteins; some may behave as activators and others as repressors in establishing the intricate pattern of LR asymmetry.

How do mutations in *ZIC3* in humans and an axonemal dynein in mice produce such similar phenotypes? Again, flies may be informative. A kinesin motor molecule, costal2, which interacts with cytoskeletal microtubules, forms a complex with Ci[18,22]. It is through interaction of the Costal2–Ci complex with the microtubular network that Ci is maintained in either an activated or a repressed form (Fig. 2*b*). Structural analysis of a kinesin protein demonstrated that certain positively and negatively charged amino-acid residues are critical for microtubule association[23]. It is therefore notable that the mutation in the axonemal dynein heavy chain causing the *iv* phenotype converts a conserved negatively charged glutamic-acid residue to a positively charged lysine[4]. Axonemal dyneins also share the ability of kinesin motors, such as costal2, to interact with microtubules. By analogy to the Ci–Costal2 interaction with microtubules in flies[24], a model is proposed in vertebrates, in which the axonemal dynein physically interacts with Zic3 and the microtubular network to either activate or repress Zic3-mediated transcription of *nodal* or *lefty* to establish LR asymmetry (Fig. 2*b*). On the basis of this model, mutations in either the gene encoding dynein or *ZIC3* would result in phenotypes similar to those seen in the *iv/iv* mouse and in human heterotaxy syndrome, respectively. A condition known as Kartagener's syndrome, which is characterized by *situs inversus*, is often asymptomatic, except for defects secondary to absence of ciliary dynein arms[25]. Although ciliary dynein is distinct from axonemal dynein, it may also play a similar role in regulating a critical member of the LR signalling cascade.

What lessons might we learn from abnormalities in LR asymmetry seen in humans? In both *situs solitus* and *situs inversus*, there is an orderly and consistent arrangement of the visceral organs along the LR axis, albeit a reversal in *situs inversus*. This is consistent with a model in which an asymmetric molecular cascade exists well before individual organs form, and this can initiate a coordinated plan in either direction. In one family described by Gebbia *et al.*, females heterozygous for the X-linked *ZIC3* mutation had either *situs solitus* or *situs inversus*, whereas hemizygous males had *situs ambiguus*, suggesting that the two conditions have overlapping pathways[2]. Two distinct forms of *situs ambiguus* (heterotaxy) are seen in humans: one represents predominant bilateral

right-sidedness (asplenia type), whereas the other has more properties of bilateral left-sidedness (polysplenia). This observation suggests that in the absence of precise asymmetry of LR signals, *situs* is randomized, but that right- or left-sided signals may be superimposed upon the randomness of lung-, heart- and gut-sidedness. It will be interesting to determine whether bilateral presence or absence of *nodal* or *lefty* correlates with either the asplenic or polysplenic forms of heterotaxy.

The discovery of mutations in a dynein protein and a transcription factor, both expressed early in midline structures, finally provides a framework in which to consider the pathogenesis of human diseases of LR asymmetry. As with most important discoveries, the reported findings raise many new questions. Will targetted mutations in mice that mimic the mutations in the genes encoding dynein and Zic3 confirm a cause-and-effect relationship? Do Zic3 and axonemal dynein physically interact as Ci and Costal2 do in flies? Can Zic3 directly influence the expression of nodal or other asymmetric morphogens? Finally, is the microtubule-associated dynein responsible for tethering, sequestering or trafficking of critical members of the LR asymmetry cascade? Just as many organisms have contributed to our current understanding, further knowledge will be dependent upon facile intellectual and experimental movement between multiple biological models. □

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Advances fuel Alzheimer's conundrum

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Alzheimer's disease (AD) is a devastating disorder that shackles the minds of it victims in confusion. With ever-growing troops of scientists and technological advances, the field seems poised for a major breakthrough that will lead directly to prevention and/or effective treatment of the disease. Recent advances made on several molecular fronts of the war against AD have, however, brought home the point that an advance of knowledge can result in breaches in prevailing hypotheses. Two fundamental events that occur in AD are the accumulation of insoluble fibrillar aggregates of amyloid β-peptide (Aβ) and the degeneration and death of neurons in the hippocampus and portions of the cerebral cortex, brain regions that function in learning and memory processes (Fig. 1*a*). Two important advances in understanding these events include the identification of the β-amyloid precursor protein (APP) as the source of Aβ and demonstrations that mutations in the *APP* gene are causally linked to a small percentage of cases of inherited early-onset AD[1]. Cell-culture and animal experiments suggest that aberrant proteolytic processing is critical, that it generates neurotoxic forms of Aβ and simultaneously reduces production of a neuroprotective secreted form of APP (sAPPα; ref. 2). Additionally, a risk factor has been identified—people with the 'ε4' isoform of apolipoprotein E (APOE) have an increased risk of developing late-onset (sporadic) AD[3]. Because apoE is involved in cholesterol transport and the ε4 allele increases the risk of atherosclerosis, this finding immediately suggested a vascular component to the pathogenesis of Aβ deposition and neuronal degeneration in AD. Surprisingly, the latter possibility has not been rigorously pursued; rather, investigators have been testing hypotheses that invoke direct effects of APOE on APP processing, Aβ fibril formation, or neuronal plasticity and survival. *In vitro* studies revealed that apoE can indeed affect Aβ fibril formation, although the results from different laboratories have been contradictory[2,3].

Now, on page 263 of this issue, Bales *et al.*[4] provide compelling *in vivo* evidence that APOE promotes Aβ deposition in the brain[4]. These investigators crossed mice with a targetted disruption of *Apoe* (Apoe null mice) with transgenic mice that express an AD-linked mutated form of human APP (PDAPP) and normally exhibit progressive deposition of Aβ in plaque-like structures in the hippocampus and cerebral cortex. The authors report that the progeny from this cross have a marked reduction in Aβ deposits—in fact, levels of APP and Aβ in these mice were normal, suggesting that Apoe acts primarily by promoting Aβ aggregation in the brain. Although the authors did not perform a 'replacement' study in which *Apoe* was reintroduced into the *PDAPP/Apoe* null mice, their data strongly suggest that Apoe promotes Aβ fibril formation.

These findings, while remarkable, defy expectation in several ways. First, studies of mice lacking Apoe have indicated that synaptic degeneration and neuronal death occur as these mice age[5] and have provided evidence for increased levels of oxidative stress and neurodegeneration after traumatic brain injury[6]. Although Bales and co-workers did not perform analyses of synaptic density and cell loss, their data suggest a dissociation between Aβ deposition and neuronal degeneration; their mice had reduced Aβ levels and increased neuronal degeneration. Second, the APOE iso-