Twirl Those Organs into Place

Getting to the heart of how a heart knows left from right

By JOHN TRAVIS

The ambulance brakes to a hard stop outside the doors of the emergency room, and paramedics rush to transfer a child critically injured in an auto accident. As the physicians struggle to stop internal bleeding, an X ray reveals a surprise. The liver and spleen aren't where the textbooks say they should be. Each is on the wrong side of the body.

That's one of the dramatic moments in an episode of *ER*, the nation's most popular television show. The bizarre plotline draws from reality. As many as 1 in 8,500 people have the normal left-right placement of their organs flip-flopped. By itself, this condition, called *situs inversus,* rarely poses any medical problems.

Nonetheless, the oddity shines light on the issue of how a growing embryo, which starts as a simple ball of cells with no asymmetries, learns its left from its right. How does the body shift the heart toward the left side of the chest, while its aorta loops to the right? What mechanism gives three lobes to the right lung, while the left has only two, apparently offering more room for the heart?

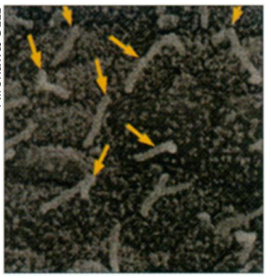
As the *ER* episode dramatically illustrates, such asymmetry persists farther down the body: The stomach and spleen normally fill the left side of the abdominal cavity, the liver and gall bladder the right, and the intestines run from right to left.

Over the past several years, developmental biologists have begun to address the origin of left-right asymmetry. Through studies of chick, frog, and mouse embryos, they've found a handful of genes that are more active on one side or the other of the early embryo (SN: 7/26/97, p. 56).

Yet scientists believe that the asymmetric expression of those genes merely reflects an earlier event in which the embryo began to distinguish left and right. That original break in symmetry is what investigators are eager to understand.

Some of them have speculated that an embryo derives its first knowledge of left and right from an asymmetrically shaped molecule that lines up along the embryo's other two axes, the head-tail axis and the back-front axis. Imagine placing an F-shaped molecule on your chest. As long as it is positioned consistently in regard to the other two axes, the arms of the F will distinguish between the left and right sides of your body.

Several studies of mutant mice, along with a dose of medical history, now offer a seemingly different explanation: In a key part of the embryo, the twirling of cilia, hairlike extensions on cells, may generate a one-way flow of molecules that ultimately lets the developing organism tell its left and right sides apart.



Hirokawa/Cell

A highly magnified image of the cilia (arrows) on mouse nodal cells.

"It's an incredibly appealing model," says Cliff Tabin of Harvard Medical School in Boston, who studies left-right asymmetry in chick embryos.

Ambiguous connections between cilia motion and internal organs have intrigued scientists for more than 2 decades. Consider the case of the not-so-identical twins. Last year, Peadar G. Noone, a pulmonologist at the University of North Carolina (UNC) at Chapel Hill, examined two sisters who were identical twins, as confirmed by DNA tests. Yet, as chest X rays made clear, "one had normal-placed organs, and the other had mirror-image organs," says Noone.

If the two women have an identical set of genes, including presumably the same mutations, what accounts for such a dramatic difference? A possible clue rests in the reason Noone and his colleagues were seeing the twins. Both women suffer from chronic middle ear, sinus, and lung problems, which the physicians finally attributed to a condition called primary ciliary dyskinesia.

Many tissues in the body sport cilia, most of which whip back and forth. The constant beating of such cilia in ears and the respiratory tract normally plays a crucial role in clearing mucous and infectious organisms. In the twins, however, these cilia were motionless. Noone concluded that having defective cilia had somehow thwarted the mechanism by which the body guarantees the normal placement of organs.

Since the 1930s, physicians have recognized that people plagued by sinus and respiratory tract problems are more likely to experience *situs inversus.* It wasn't until 1976, however, that a researcher put forth cilia as an explanation.

That year, Björn A. Afzelius of Stockholm University published a description of four infertile men. Afzelius had noticed that the men's sperm had defective flagella, the whiplike tails that are essentially modified cilia. Without working flagella, the sperm couldn't move. Three of the men also complained of chronic sinus and respiratory problems, suffering frequently from colds, ear infections, and pneumonia.

When Afzelius examined tissue from the bronchial tubes of one of the men, he didn't see any beating of the cilia. In fact, the cilia looked abnormally dense and lacked structures called dynein arms that normally allow the cilia to move.

Afzelius concluded that the men's problems stemmed from an inability of various cilia to move. He also noticed that three of the men had *situs inversus,* and he hypothesized that other immotile cilia were also responsible for that oddity.

"I postulate that cilia on the embryonic epithelia have a certain position and a fixed beat direction (in normal embryos), and that their beating somehow is instrumental in determining the visceral situs [the placement of organs]," Afzelius wrote in Science in 1976.

Afzelius' theory languished in obscurity for the next 2 decades, largely because scientists could find no evidence of motile cilia in embryos at the time they learned left from right. But in 1994, Kathleen Sulik of UNC-Chapel Hill and her colleagues found moving cilia on cells in the node, a small region of the early embryo known to help determine the overall body plan of an animal.

Another group failed to see such movement, however, prompting most researchers to attribute the motion seen by Sulik's team to random currents in the medium bathing the cells. "We sort of backed off and thought we were stupid," laughs Sulik.

Other aspects of the nodal cilia also encouraged the belief that they were unlikely to move. Unlike the beating cilia of

adult tissues, which have a so-called 9+2 structure (a circle of nine rods surrounding two center rods), the nodal cilia lack the two central shafts. Such 9+0 cilia have never displayed motility in adult tissues. "We've literally looked for hundreds and hundreds of hours, using time-lapse video, and they don't move at all," says Samuel S. Bowser of the Wadsworth Center in Albany, N.Y.

Still, the node appeared to offer an excellent location for the embryo to learn left from right. In the past few years, scientists have found that some of the earliest left-right asymmetries in mouse gene expression occur in the node.

Late last year, a research group led by Nobutaka Hirokawa of the University of Tokyo offered evidence that Sulik and her colleagues shouldn't have doubted themselves. The Japanese scientists reported in the Dec. 11, 1998, CELL that the nodal cilia of mouse embryos do move, although not in a back-and-forth manner. Movies taken through a powerful microscope revealed that the cilia twirl in a counterclockwise rotation.

"I was very surprised when I read that report," says Bowser.

Hirokawa's team stumbled across the unexpected finding when they created a strain of mice with a mutation in the gene for a protein that helps move molecular cargo along cellular filaments called microtubules. The mutant mice experience a variety of development abnormalities and die before birth. What caught the scientists' attention, however, was that the normal left-right asymmetry of the fetal heart was frequently perturbed.

Following up on that finding, the researchers discovered that the nodes in their mutant mice had no cilia at all. That led them to closely examine the cilia in normal mice and discover the circular motion. By placing buoyant, fluorescent beads in the liquid within which they study nodal cells, Hirokawa and his colleagues then showed that the cilia generate a leftward current, or "nodal flow."

While a circular motion by cilia would seem to generate equal left and right currents, the researchers speculate that the triangular shape of the node and other features of this embryonic region combine to create a one-way stream.

In addition, they suggest that this nodal flow initiates left-right asymmetry by shifting chemical signals for development to one side of the embryo. "In this model, a putative secreted factor is concentrated to the left by the nodal flow, and this then triggers the downstream signaling cascade of left-defining genes," the investigators said.

The results from Hirokawa's group have forced some researchers to rethink their own findings. Two years ago, collaborating groups led by S. Steven Potter of the Children's Hospital Research Foundation in Cincinnati and Martina Brueckner of Yale University announced the discovery of a gene at the core of left-right determination in the mouse embryo (SN: 11/15/97, p. 311).

The scientists had been studying strains of mice in which almost half the newborn rodents had *situs inversus*. The rodents, the scientists found, possessed a mutation in the gene for a dynein. Such proteins come in two distinct forms. One version forms the dynein arms, whose motion whips 9+2 cilia back and forth. The second acts as a motor that helps transport cargo along microtubules.

Even though the dynein that they uncovered looked like the type that move cilia, the investigators initially theorized that their dynein interacts with microtubules to define left and right. Like most other biologists, they firmly believed that the nodal cilia were immotile.

Since then, however, Potter and his colleagues have confirmed that the nodal cilia from normal mice do twirl and that this motion generates a leftward current. "It's not an easy thing to document, but you can clearly see the cilia spinning around," says Dorothy M. Supp of the Cincinnati Shriners Hospital, who participated in the work.

Of even more possible significance, in mice with the dynein mutation, the nodal cilia are present and appear normal but don't move. "They're frozen in rigor on the node cells," Brueckner revealed in June at the Society for Developmental Biology meeting in Charlottesville, Va.

Such a finding supports the idea that the nodal flow created by cilia plays a role in defining left-right asymmetry. Presumably, an alteration of this flow in the dynein-lacking mutant mice somehow results in a random choice between normal organ placement and *situs inversus*.

Still, many scientists are cautious about jumping to such conclusions. "You have to be jolly careful that the change in the cilia isn't reflecting a change in the molecule [the dynein] acting somewhere else," notes Lewis Wolpert of the University College London.

Biologists are now examining other species, such as frogs, zebrafish, and chicks, to determine whether those animals possess nodal cilia and whether those cilia twirl. It's still unclear whether nodal flow represents the initial break in left-right symmetry during development. "We're going to have to figure out if this is the thing that starts the ball rolling or not," says Joseph H. Yost of the University of Utah in Salt Lake City.

Paul Overbeek of the Baylor College of Medicine in Houston suggests that the leftward flow somehow reflects an inherent asymmetry of cilia. He notes that the cilia consistently line up along the body's other two axes, as such a candidate should. Moreover, as a result of the way the rods are arranged in the 9+0 structure, cilia normally have a handedness, much as a spiral staircase ascends clockwise or counterclockwise.

In chicks, however, there's some evidence that the node reinforces an earlier origin of left-right asymmetry. Data from several groups suggest that the two sides of an embryo have different genes in action even before the node forms.

"The node is an extremely important relay station in all of this but may not be where the initial [left-right] calculation is done," contends Mark Mercola of Harvard Medical School.

An experiment on another mouse strain may shed further light. In this strain, called *inv* for inverse, newborn rodents display *situs inversus* about 85 percent of time. This finding has prompted investigators to speculate that nodal cilia in these mice spin in reverse.

Hirokawa has looked at the cilia in *inv* mice, but declines to discuss his group's results because they're still being reviewed for publication. If the cilia in *inv* mice do not spin backwards, ideas about left-right asymmetry may be thrown back into confusion.

When scientists do finally unravel how embryos learn to tell right from left, a fundamental question may still keep them awake at night. Why do normal embryos so consistently choose one orientation for their internal organs? If flipping the left and right axes causes no obvious problems, why isn't there a 50-50 split between people with normal placement and those with *situs inversus*?

No one has a good answer, admits Wolpert. He suggests that settling on a consistent arrangement of internal organs is more reliable than choosing sides each time. For a growing embryo, placing its heart in the right place—on the left side—is serious business after all. □

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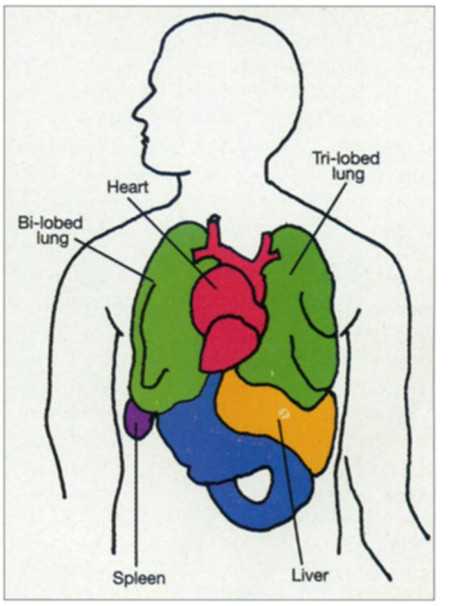

People with situs inversus *have their internal organs flipped left to right. The heart rests more on the person's right instead of left, and its aorta loops left instead of right. The lung with three lobes, normally on the right, is on the left. The liver and spleen also switch sides.*