DEVELOPMENTAL BIOLOGY

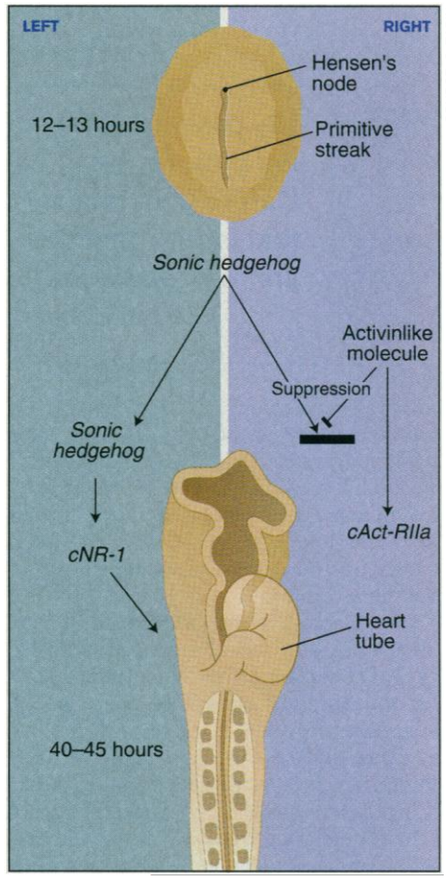
Embryos Travel Forking Path As They Tell Left From Right

Adults sometimes have trouble telling right from left, but a vertebrate embryo, from the time it's just a few hours old, seems to have no such difficulty: It manages to place internal organs, such as the heart and liver, on the correct side of its developing body. The origin and nature of the directional signals that make this possible, however, have mystified biologists for many years.

Now scientists have defined some of the early steps embryos take toward left-right asymmetry, identifying a cluster of genes involved in producing this biological "handedness" in chick embryos. In the 8 September issue of *Cell*, a team of developmental biologists led by Cliff Tabin of Harvard Medical School (HMS) in Boston reports that in the first 48 hours of development, some genes are expressed on the right side of the embryo and some on the left, and this leads to the precursor of the heart looping toward the right side. The heart tube's bend "is the first gross morphological change indicating handedness in chick embryos," explains Tabin. By manipulating the interactions of these genes, the researchers can cause the developing organ to bend randomly to either side.

The findings don't explain the ultimate origins of left-right choice, but they do give biologists their first glimpse of the interactions within and between cells that implement the asymmetric blueprint of the chick's body (*Science*, 30 April 1993, p. 679). "That's why these results are so exciting," says David McClay, a developmental biologist at Duke University in North Carolina. "The genetic pathways forming the anterior-posterior and dorsal-ventral axes are fairly well established ... but until now we've known virtually nothing about the genetic components of left-right asymmetry."

Adding to biologists' excitement, the genes Tabin's group has fingered are "not new customers," in McClay's words, "but the same old players used in different ways." The first gene identified in this pathway, for example, is *sonic hedgehog* (*Shh*), which helps developing vertebrates' limbs tell anterior (head) from posterior (tail). Its appearance in left-right patterning indicates that the list of molecules used to establish the vertebrate body's three axes "is even more limited than we might have expected," McClay says. Other scientists caution, however, that in some animals, such as mice, some of these genes don't appear to have left-right roles, so the eventual picture may be a complex one.



SOURCE: C. TABIN

Right road to a heart. Asymmetric expression of several genes in a chick embryo leads to a right-handed heart.

Still, it was the genes that separate head from tail in chicks that started Tabin's team down this left-right path. They and other groups had first described the role of *Shh* in the chick in 1993. But during these experiments, the researchers had noticed that after a certain stage in embryonic growth, messenger RNA from *Shh* was expressed only on one side of a site called Hensen's node. The node is an area at one end of the primitive streak—a thickening in the embryo's outer layer that bisects the embryo—where many important developmental signals are dispatched. While the observation was "intriguing," questions about *Shh*'s role in limb patterning had higher priority at the time, Tabin says.

Michael Levin, a doctoral student in genetics at HMS, volunteered to dig deeper into the phenomenon last year. He began by charting the expression of various genes around Hensen's node. Claudio Stern, a developmental biologist at Columbia University and a co-author of the *Cell* paper, had sent word that about 18 hours after incubation, when the embryo starts to develop, messenger expression of a gene called *cAct-RIIa* suddenly turns on—but only on the side of the node that ultimately becomes the right side of the chick. The gene is thought to be a receptor for activin, a hormone known to play a role in tissue differentiation. That piqued Levin's interest, and when he found that right-sided expression of *Shh* ceases at precisely the same time, he wondered if there was a connection.

Things were happening on the left side, as well. At about 24 hours after incubation, the investigators found, messenger expression of *Shh* on the left side of the primitive streak shuts down, and expression of a gene called *chicken nodal-related 1* (*cNR-1*) begins.

The implication was that the scientists had found part of a diverging, asymmetric pathway: Activin or an activinlike molecule might be triggering *cAct-RIIa* expression while also suppressing *Shh* expression on the right side. On the left, unsuppressed *Shh* might trigger *cNR-1* (see diagram). The question was whether these signals were indeed related to one another, and if they actually have an influence on internal anatomy. To answer it, the researchers needed direct evidence.

They soon got some. Tabin's team (which also includes HMS researcher Randy Johnson and Michael Kuehn at the National Institutes of Health) report in *Cell* that they soaked tiny acrylic beads in activin—a presumed part of the right-side path—and implanted the beads on the left side of Hensen's node. The result: *cAct-RIIa*, normally expressed only on the right side, was expressed on the left as well. And *Shh*, usually expressed on the left, was suppressed. That in turn prevented the expression of *cNR-1*.

Eventually, these crossed signals had an effect on developing organs. In some 18-hour-old embryos, the researchers implanted cultured pellets of cells expressing *Shh* on the right side of the node; in others they implanted beads soaked in activin on the left. After another day of growth, the heart tube, which normally zigs to the right, zagged left in 50% of the embryos—a random pattern. Presumably the abundant *Shh* expression on the right made moot the usual shut-off signal, and activin on the left upset the balance there.

The finding provides researchers with a loose scaffolding for the future placement of other genes and proteins involved in the unfolding asymmetry of the organism, says Joseph Yost, a developmental biologist at the University of Minnesota. "There might still be 10 or 20 unknown steps in the cascade that Levin *et al.* have identified, but the nice thing is that this narrows down some of the intermediate events," he says.

Some biologists, however, note that even

these events haven't been narrowed quite enough. Nigel Brown, a teratologist (specializing in the study of congenital malformations) at St. George's Hospital Medical School in London, and Lewis Wolpert, a developmental biologist at University College, London, are enthusiastic about the advance, but say that a 50/50 ratio of heart handedness can result from other influences, such as heat shock, and that Tabin's group should have tried for a 100% flip by implanting wrong-sided activin and *Shh* in the same embryos. Tabin responds: "We think the results are unlikely to be artifactual, because control implants did not cause randomization. However, the experiments Lewis and Nigel suggest are indeed being done."

Brown also notes that the search for equivalent genetic cascades in other organisms could run into "several potential difficulties." Mice lacking activin receptor genes, for example, have normal asymmetry, suggesting that mammals may not require this protein for left-right specification, he and Wolpert note.

Still, Wolpert is confident the researchers are on the right track. "Right now there are puzzles," he says, but left-right specification in vertebrates "will probably all be the same in the end." For an asymmetrical story, that would be a strikingly even-handed conclusion.

–Wade Roush

IMMUNOLOGY

Long-Sought H-Y Antigen Found

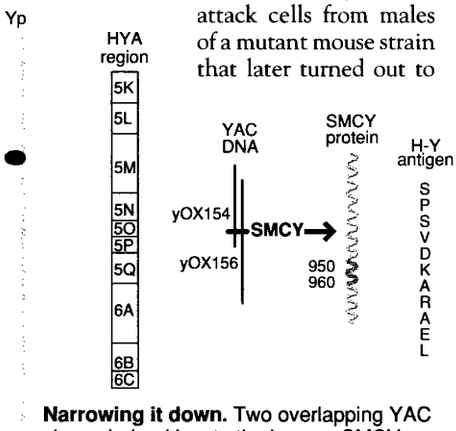
Ever since 1955, transplant surgeons and immunologists alike have been perplexed by a mystery. Although males seem to tolerate transplanted female tissues very well, females sometimes reject transplants from males, even when the tissues are closely matched immunologically and genetically. "It's something that's been driving immunologists crazy for many years," says immunologist Victor Engelhard of the University of Virginia, Charlottesville. But no longer.

Two reports, one in the 24 August issue of *Nature* from a team led by Elizabeth Simpson of the MRC Clinical Sciences Centre at Hammersmith Hospital in London and Michael J. Mitchell of INSERM in Marseilles, France, and the other on page 1588 of this issue of *Science* from Engelhard, Els Goulmy of Leiden University Hospital in the Netherlands, and their colleagues, have now solved at least part of the mystery. Both groups have found an elusive factor that makes male tissue unacceptable to females, the Simpson-Mitchell team in mice and the Engelhard-Goulmy team in humans. It turns out to be a short peptide encoded by a segment of a gene called *SMCY*, which is found on the Y chromosome of males.

Immunologists are relieved, says Polly Matzinger, an immunologist at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland. "Forty years of failure makes people hungry," she declares. But the quest may not be over, as more H-Y antigens may lie waiting in the wings. Even so, the discovery means that researchers can begin to develop ways to avoid or neutralize H-Y rejections and, as a result, allow more frequent male-to-female transplants. In fact, the prospect that H-Y antigens might be clinically useful delayed publication of the *Science* paper as the biotech firm Promega Corp. of Madison, Wisconsin, sought to protect its stake in the finding.

Immunologists learned early on that the antigen at fault in the organ rejection was encoded by a Y chromosome gene, because the problem occurred in inbred mice that were genetically identical except for the fact that the males had a Y chromosome while the females didn't. That's why they dubbed it the H-Y antigen, for histocompatibility antigen from the Y chromosome. But for 2 decades researchers made little headway in further narrowing down H-Y's identity or the gene's location, primarily because they were unable to come up with antibodies to the antigen that could help them in their search. As immunologists learned in the mid- to late 1970s, antigens like H-Y are formed intracellularly and, as a result, do not generally trigger antibody production. Instead, proteins encoded by genes of the major histocompatibility complex (MHC) display the antigen peptide on the cell surface, where it draws attack by the immune system's T cells.

At about the same time, geneticist Simpson established T cell clones that specifically killed cells carrying the H-Y antigen, thereby providing a tool that would help in the hunt. Indeed, in the late 1980s, the cells provided a critical clue when Simpson found that they did not attack cells from males of a mutant mouse strain that later turned out to be missing a piece of the short arm of the Y chromosome. This showed that the H-Y antigen gene was located in the deleted section, thereby narrowing the search down to about 900 kilobases of DNA.



Narrowing it down. Two overlapping YAC clones helped locate the human *SMCY* gene, which codes for the H-Y antigen.

SOURCE: WANG *ET AL.*

By that time, geneticist Colin Bishop, who was then working at the Pasteur Institute in Paris, and his colleagues, including Mitchell, who was in Bishop's lab at the Pasteur, had begun a systematic search through the DNA of the deleted Y chromosome region. But it was not until 1994, after Bishop had moved to Baylor College of Medicine in Houston, that he and his colleagues found a likely looking gene: *SMCY*, which stands for "selected mouse cDNA on Y." Unlike earlier candidates, which were active only in the testis, this gene was expressed in all kinds of tissues. Also, even though *SMCY* has a counterpart, *SMCX*, on the X chromosome, the two genes are only 82% identical—different enough that peptide fragments of the male protein could look foreign to a female immune system.

Simpson and her colleagues built on that work, she says, by combing the *SMCY* DNA for the specific sequence coding for the peptide: "We approached it scissorlike—immunologically and genetically." In collaboration with Mitchell, her group began putting ever smaller pieces of *SMCY* DNA into cells taken from female mice and testing those cells to see if they were attacked by the H-Y antigen–specific T cells. They also compared the DNA pieces that elicited a reaction with parallel sections of *SMCX* to identify the differences. By June, the Simpson-Mitchell team had homed in on their H-Y antigen, an eight-amino-acid peptide located near one end of the SMCY protein. "It's a great relief and pleasure," Simpson says.

The Engelhard team took a totally different tack in their search. Instead of dissecting a promising gene, they pursued the peptide directly. Engelhard and his chemist collaborator Donald Hunt, also at the University of Virginia, had developed a technique for pinning down hard-to-find peptide antigens by pulling them off the MHC molecules that display them on the cell surface and then sorting and testing the peptides. Engelhard says that after he was contacted earlier this year by Goulmy, who had worked with Simpson pursuing H-Y in both mice and humans, "we all agreed that H-Y, because of its history, should be our next [target]."

By June, the team had identified a candidate peptide. When they then searched the databases for genes that could encode a peptide with that sequence, the best they came