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## Learning How Organs Tell Left From Right

From all outward appearances, the human body is symmetrical. If one were to divide the body into two halves, for example, each side would have a single arm and leg. But a look inside at the internal organs shows that there is not perfect symmetry throughout the body. The heart and spleen normally reside on the left side and the liver and gallbladder are on the right. Furthermore, the organs themselves are asymmetrical.

Understanding how genes control the shape and spatial orientation of organs is one of the goals of developmental biologists. In the mouse, as in other vertebrates, cells begin receiving and responding to instructions about their positional fate during the earliest stages of development, when the embryo resembles little more than a flat sheet of cells. Two proteins in particular, called Nodal and Pitx2, are produced predominantly on the left side of the mouse embryo and they appear to direct the growth of organs in a way that is appropriately "left-sided." Understanding how these two proteins interact may allow scientists to understand a great deal more about how organs are segregated into left-side and right-side.

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— Michael G. Rosenfeld

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In attempting to unravel the genetic program that determines left/right orientation, Howard Hughes Medical Institute (HHMI) investigator Michael Rosenfeld and colleagues at the University of California, San Diego, and The Salk Institute, engineered knockout mice that lacked the *Pitx2* gene. The mice exhibited a number of developmental abnormalities, including inappropriate position of the heart and lungs.

Humans with one defective copy of the *Pitx2* gene have a constellation of problems called Rieger syndrome, the signs of which include irregularly shaped eyes, lack of tooth growth, craniofacial deformities, and, more rarely, problems with growth hormone production.

Rosenfeld's team found that *Pitx2* knockout mice had defective growth in their teeth and pituitary glands, the source of growth hormone. "Cell proliferation stops. There is a failure to progress past the initial determination of organ identity," Rosenfeld said.

Summarizing the results of these studies, which are published in the September 16, 1999, issue of the journal *Nature*, Rosenfeld said, "the work shows that the control of the left/right orientation of organs is more complicated than we thought."

Researchers knew that Nodal is a signal-carrying protein, and *Pitx2* is a transcription factor protein that turns on other genes. Once the Nodal signal arrives, it induces *Pitx2* to help implement its directives. One testable issue in the current study, says Rosenfeld, "was to determine whether *Pitx2* implements the entire Nodal program or whether Nodal acts via additional targets."

The results of earlier experiments that attempted to untangle the functions of Nodal and *Pitx2* were intriguing, but did not produce a clear answer. When researchers added Nodal or *Pitx2* to the right side of a mouse embryo, for example, the right side developed as a mirror image of the left. In other experiments, researchers added extra Nodal or *Pitx2* to embryos only to find that the two proteins had similar effects. And knocking out the *Nodal* gene alone resulted in either mirror-image or randomized organization of multiple organs.

Given these results, Rosenfeld says he would have expected the knockout of *Pitx2* to cause the same sort of organ abnormalities reported by scientists who performed the Nodal knockout experiments.

The actual result was a mixed bag, but it appears that *Pitx2* is crucial in determining the "leftness of the lung," say Rosenfeld and his colleagues. Without *Pitx2*, both sides of the lungs of the knockout mice looked like the right side of a normal lung. The left side of a normal pair of lungs is usually smaller to accommodate the position of the heart.

Studies of the heart orientation in the *Pitx2* knockout mice proved less conclusive. In the *Pitx2* knockout mice, the heart was located on the right side of the body, not in its normal location on the left side. Despite its improper location, however, the direction of cardiac looping was normal.

"Everyone might well have predicted the result would be the same as after removal of *Nodal*," said Rosenfeld. "But now we know that this single transcription factor in the pathway does not account for the whole of left/right asymmetry."

Rosenfeld is now searching for other transcription factors that respond to Nodal signals. These transcription factors may be involved in a condition called situs invertus, which affects approximately 1 in 10,000 humans and gives rise to a mirror-image organization of the internal organs. In many cases the disorder has no adverse consequences, though it can be associated

with heart abnormalities.

Rosenfeld also hopes to begin identifying genes that are switched on by Pitx2 when organs grow. Such genes are likely to provide additional insight into understanding left/right determination, he says.