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RESEARCH NEWS

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Gene Orchestrates Heart Chamber Development

Early in development, the vertebrate heart is transformed from a narrow tube into a powerful four-chambered pump. Researchers have now found a gene that influences formation of the chambers of the heart. The discovery also provides a new set of tools to probe how this crucial developmental step occurs.

Zheng-Zheng Bao, a postdoctoral fellow in HHMI investigator Constance Cepko's laboratory at Harvard Medical School, identified *Irx4* (for *iroquois*-related homeobox gene), a gene expressed only in the ventricles of embryonic chicken hearts.

> "Finding *Irx4* provides an important lead for the discovery of other genes involved in heart development."
> **— Constance L. Cepko**

"This is the first gene that is the right type and is expressed early enough to play a key regulatory role in setting up the chambers," Cepko says. She and her colleagues published their research in the February 19, 1999, issue of the journal *Science*.

All vertebrate hearts have two types of chambers—atria and ventricles, which pump blood into and away from the heart, respectively. In vertebrate embryos, the developing heart first resembles a tiny tube, which folds into four chambers as it grows. "How these different chambers form during development is not known," says Cepko.

Heart muscle in the atria and ventricles has different contractile properties that can be traced to different forms of the protein myosin. In an attempt to understand the early stages of cardiac development, researchers have mapped the expression patterns of the myosin proteins. They know that atrial myosin heavy chain-1 gene *(AMHC1)* is expressed only in the atria, for example, while the ventricle myosin heavy chain-1 gene *(VMHC1)* is restricted to the ventricles. It has not been clear how expression of these genes is restricted to one chamber or the other, Cepko says.

A developmental neurobiologist who is noted for her work on the visual system, Cepko says that her team branched into cardiac development by accident. While studying the family of *iroquois* genes that regulate pattern formation in the eyes of *Drosophila,* Cepko and colleagues wondered if similar genes in other animals played an analogous role. They were not surprised to find that *Irx4* is expressed in the brain and eye of chick embryos, but, Cepko admits, they became more curious when they found that gene showed up in only one type of the chambers of the heart.

A homeobox gene, *Irx4* influences the expression of many other genes. "Homeobox genes frequently tell a whole chunk of tissue to organize or tell a cell to become a certain cell type," Cepko said. Since the studies showed that *Irx4* is expressed only in the ventricles, Bao and Cepko reasoned that it might guide the fate of ventricular precursor cells.

To test this hypothesis, Bao and Cepko collaborated with Benoit Bruneau and HHMI investigators Jonathan Seidman, also at Harvard Medical School, and Christine Seidman at Brigham and Women's Hospital. They showed that the first hint of *Irx4* expression occurs when the embryonic heart is still a tube. During this time, expression is restricted to the middle portion of the tube, which forms the ventricles.

The group also found that *Irx4* regulates two myosin genes found in the heart, *AMHC1* and *VMHC1*. Before *Irx4* is expressed in the chick embryo, both myosin heavy chain genes are expressed throughout the developing heart. But once *Irx4* is "turned on," *AMHC1* is only expressed in the atria and *VMHC1* in the ventricles.

Further probing the role of *Irx4,* the investigators inserted an extra copy of the gene into the atria of developing chick embryos, and saw that the expression of myosin genes was perturbed-the *VMHC1* gene was turned on and *AMHC1* expression was reduced markedly. In another experiment, the investigators turned off *Irx4* in the ventricles, and, in turn, showed that *AMHC1* was activated and *VMHC1* was turned off. "*Irx4* activity is required to keep those proteins in their respective chambers," explains Cepko.

The researchers also found that a similar gene in mice shows the same type of expression pattern in ventricles. Since mice and chickens have such similar genes, Cepko says that she expects to find some close homologues in other species, including fish, humans and frogs.

She does not, however, expect *Irx4* to be *the* master regulator of heart chamber development. "This is an opening into how the heart develops, how the different chambers form. Other genes are surely involved in this complex process," she says. "Finding *Irx4* provides an important lead for the discovery of other genes involved in heart development."