DEVELOPMENTAL BIOLOGY

Connexin Knockout Provides a Link to Heart Defects

If anything proves the adage "You don't know what you've got 'til it's gone," it's the knockout mouse. Such mice, created by using genetic engineering techniques to inactivate, or "knock out," specific genes, have become a boom industry for researchers who want to pinpoint the role of genes whose functions in the animal are unknown. The most recent case in point: A Canadian research team has produced the first knockout of a gene encoding a connexin, a member of a family of proteins that form the cell communication links known as "gap junctions" (see p. 1831).

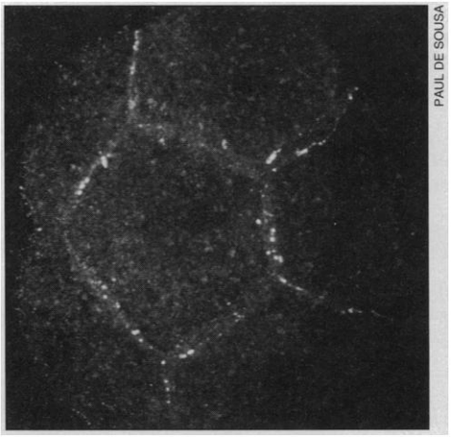
The result turned out to be both more—and less—than the researchers expected. On the positive side, it may provide a new model for understanding congenital heart defects, such as pulmonary stenosis. Every year in the United States about 32,000 children are born with heart defects, and this is one of the most common. On the other hand, researchers were surprised to find that knocking out this particular connexin, known as connexin43 (Cx43), had less catastrophic effects on fetal mice than they expected—which may lead researchers to re-evaluate some of their ideas about the role played by connexins in early embryonic development.

The fact that the disruption was fairly mild surprised even the Canadian team itself, which was led by developmental biologists Janet Rossant of Mount Sinai Hospital in Toronto and Gerald Kidder of the University of Western Ontario in London. In fact, says Rossant, the team chose to knock out the Cx43 gene because that protein is expressed in mouse embryos with as few as eight cells, and researchers assumed a protein expressed so early in the embryo's history must be crucial for normal development.

"We wanted to knock out the main gap junction in these animals to study signaling in early embryos," Rossant explains. Gap junctions, water-filled channels that connect the cytoplasmic compartments of two coupled cells, are thought to be important conduits for the transmission of many kinds of cell-to-cell signals, including those that tell cells what developmental paths to follow.

But the embryos lacking the Cx43 gene didn't die immediately, as the researchers thought they might. "Since this protein is expressed so early in development, one would have thought a priori that [it] would be necessary for survival. But they found out that the organism did not only survive, but made it to birth," says Norton Gilula of the Scripps Research Institute in La Jolla, California, whose group, along with Kidder's, showed that the Cx43 gene is expressed in early embryos.

The knockouts were not normal, however. Shortly after birth, the animals died with labored breathing and blue coloration,



PAUL DE SOUSA

Bridging the gap. Connexin43 *(bright stain)* can be seen in this eight-cell mouse embryo.

suggesting they were unable to get oxygen from their lungs into the bloodstream.

Autopsy of the affected mouse pups showed why: The right ventricle, the chamber of the heart that pumps blood through the pulmonary artery to the lungs, was abnormal. The region where the pulmonary artery exits the heart was filled with overgrown tissue that obstructed blood flow, preventing blood from reaching the lungs. Such a defect "would pose no problem," Rossant says, until birth because in utero the animals could get oxygen from the mother's placenta, but afterward it would be lethal.

The heart defects shown by the knockout mice resemble congenital pulmonary stenosis in humans, a condition in which anatomical blockage in the heart's right ventricle prevents normal blood flow to the lungs. Further evidence that Cx43 plays a role in heart development comes from Scott Britz-Cunningham, William Fletcher, and their colleagues at the Jerry L. Pettis Memorial Veterans Administration Medical Center in Loma Linda, California, who have recently linked mutations in the Cx43 gene to another congenital condition, called visceroatrial heterotaxia. Although this condition causes a spectrum of abnormalities, some very mild, it can also cause serious, even lethal, heart defects, such as the reduction of the normal four chambers to two.

What does this overall picture—mice that survive to term, with a full set of organs, including a beating heart, only to succumb at birth to a severe heart problem—tell researchers about embryonic development? One thing is that these studies are not the first to find that a connexin defect can cause a relatively specific developmental abnormality, rather than being fatal for the early embryo. About 15 months ago, two teams, one led by Jo Ann Bergoffen of the University of Pennsylvania School of Medicine in Philadelphia and the other by N. Fairweather of the University of Aberdeen Medical School in the United Kingdom, found that one form of a hereditary condition called Charcot-Marie-Tooth (CMTX) disease has been linked to mutations in the gene for another connexin, connexin32 (*Science*, 24 December 1993, p. 2039). In this case, the defect appears to be limited to the cells that form the linings around many peripheral nerves, which deteriorate in late childhood.

Exactly why the effects of the connexin knockouts and mutations are limited remains unclear. This is particularly puzzling, says Dan Goodenough of Harvard Medical School, whose lab cloned the Cx43 gene in 1987, because several groups, including his own and that of Gilula, have found that in normal animals, Cx43 is expressed throughout development in many types of tissue. Cx32 is also expressed in several kinds of tissue. Paul de Sousa, another member of the Canadian team, notes, however, that mice have genes for at least 12 connexins, and one or another of them might have been able to substitute for Cx43 in most tissue types, although apparently not in the right ventricle of the heart.

The next step, Rossant suggests, is to examine other connexins to see if their expression goes up during development to compensate for the missing Cx43. And the connexin studies won't be limited just to mice. Gap junction researchers will want to check for missing connexins in patients with congenital heart abnormalities to confirm that they have a role in these conditions as well as in CMTX. Meanwhile, at least two additional connexin knockouts are on the way, as they were announced at a meeting held in Ile des Embiez, France, in early March. Researchers expect that these will further clarify connexin activities. "The knockout animal is a very important experimental animal, because it gives you the ability to study each connexin to see what the cells [lacking it] can't do," Goodenough says. Indeed, these new tools are sure to win a warm acceptance—especially if the tantalizing links to human disease hold up.

–Amy Stone

Amy Stone is a free-lance writer based in Atlanta.