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Zebrafish May Point the Way to Mending a Broken Heart

Researchers have found that the secret to mending a broken heart— at least at the molecular level— resides within the two-chambered heart of a fish commonly found in household aquariums. The scientists showed that the zebrafish can regenerate its heart after injury, and their studies suggest that understanding cardiac regeneration in this fish may lead to specific strategies to repair damaged human hearts.

Many studies have documented that various invertebrates can regenerate vital organs. But most vertebrates and all mammals develop scarring in response to cardiac injury, with minimal regeneration of heart muscle. In an article published in the December 13, 2002, issue of the journal *Science*, Howard Hughes Medical Institute investigator Mark T. Keating and colleagues at Harvard Medical School and Children's Hospital in Boston report that the zebrafish is one vertebrate that is capable of cardiac regeneration. Two months after Keating and his colleagues removed 20 percent of the heart in zebrafish, the fish had fully regenerated the excised portion of the heart. Keating's group also reported that zebrafish with an induced mutation in a specific gene failed to regenerate heart tissue and instead developed scarring.

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— Mark T. Keating

Improved understanding of heart regeneration and the associated problem of cardiac scarring have lagged behind "for want of a genetically manipulable system to study the problem," Keating said. Very few genetically well-characterized organisms regenerate damaged heart muscle, and most scientists who study regeneration focus on fully regenerating invertebrates including planarians, or flatworms, and the *Hydra* polyps, he said. In addition, the complex and poorly understood genomes of these organisms have proven difficult for scientists to manipulate in experiments.

Researchers have studied organ regeneration in vertebrates, such as newts and salamanders, but their hearts scar when damaged. "One vertebrate organism that is different," said Keating, "is the zebrafish. It is manipulable and its genetics and genomics are better known. And it can regenerate just

about every type of organ. The zebrafish could take this field of research out of the Dark Ages."

In the zebrafish studied, Keating and his colleagues made surgical incisions in the ventral side of adult zebrafish, pushed out the two-chambered heart and then clipped off a chunk at its apex equaling about 20 percent of the total organ. After staunching the bleeding, the scientists put the hearts back in the fish. Some 80 percent of the fish survived the procedure.

To follow the healing process, Keating and his colleagues examined the hearts of the fish in the study over a two-month period. They examined the hearts under a microscope, and began to see before their own eyes how a heart begins the process of regeneration.

According to Keating, regeneration commences with a clot of erythrocytes that forms at the site of the wound. Over the next week, fibrin, the thick, static tissue that forms a mature clot. Starting a little over a week after the surgery, though, Keating observed a startling process: the heart started to grow back into its previous form.

In the first phase of this process, cardiac myofibers, or cardiomyocytes, the tissue that composes heart muscle and wall, begin to infiltrate and replace the fibrin over the wound. At one month after the surgery, a completely new heart wall forms and, based on observations of chemically labeled cells, the scientists could see that its leading edge then proliferates and expands rapidly outward until it completely reforms the missing apex of the heart. Two months after the surgery, all evidence of scarring is gone, and the size, shape and cellular activity of the zebrafish hearts appear to be no different from typical zebrafish. The regenerated heart beats just like a normal, healthy one.

Keating says that his team's observations indicate that the process of regeneration in the heart appears similar to that seen in other organs. The specialized heart muscle cells nearest the wound dedifferentiate, that is, they lose some of their specialized characteristics and are capable of cell division and migration. These stem cells then proliferate as cardiomyocytes until they have rebuilt a completed heart.

Although most studies of cardiac regeneration in vertebrates indicate that scarring is a major problem, the zebrafish continued to regenerate heart tissue with little or no scarring. Keating's theory is that while both regeneration and scarring are possible in the zebrafish, a competition takes place between the two. The regenerative mechanism rapidly overwhelms the formation of scar tissue in this case, Keating says.

To test the theory, he and his colleagues studied the process of cardiomyocyte proliferation to see what would happen when it was inhibited. The investigators looked at zebrafish with a mutation in *Mps1*, a gene that encodes a mitotic checkpoint kinase protein critical in cells that regenerate zebrafish fins. At temperatures above 33 degrees Celsius the mutant form of *Mps1* can no longer function properly and the cells stop proliferating. When the temperature is raised to that level, the fish with the mutant form of the

kinase protein cannot regenerate hearts. Instead, they produced scars at the site of the wound.

"That tells us two things," said Keating. "Cell proliferation is essential for regeneration, and there is a competition between the regeneration potential of the organism and scarring." In normal zebrafish, regeneration wins.

Keating is excited about this finding because it provides a hint that there may be a similar competitive situation at work in human hearts, which show a minimal regenerative capacity following heart trauma such as a heart attack. "If one enhances the regenerative potential in humans," he said, "perhaps one can overcome the fibrotic potential."