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Thanks be to Zebrafish

Ruth Williams

During the last 10–15 years, the tiny, tropical, freshwater zebrafish has become one of the most powerful and versatile models for studying vertebrate heart development. Three recent reports in *Nature* remind us of just how much we owe to this fishy friend of research.

The zebrafish was first developed for use as a model organism by George Streisinger at the University of Oregon in the 1970s. A common resident of household tropical aquariums, the fish soon became a common laboratory resident thanks to its hardiness and many other research-friendly characteristics.

The zebrafish is small, easy to keep, and produces large numbers of offspring on a regular basis—female zebrafish can produce up to 200 eggs per week. Such features make it perfect for genetic analysis. Its genome has been fully sequenced, large-scale mutation screenings have been carried out, and countless transgenic strains and human disease models are available. In addition, the zebrafish embryo is transparent, develops rapidly, and grows externally from the mother, allowing for easy experimental manipulation and observation. The zebrafish is, thus, close to being the ideal model organism for studying vertebrate development.

In the field of cardiovascular biology, studies of zebrafish heart development have revealed a large number of genes and pathways that are conserved with higher vertebrates and mammals. Thus, although the zebrafish is coldblooded and its heart has just a single ventricle, it serves as a great tool and starting point for comparative analysis.

One feature of the zebrafish heart that differs dramatically from that of mammals is the capacity for regeneration. In 2002, Kenneth Poss (Duke University, Durham, NC) and colleagues reported in the journal *Science* that after removing up to 20% of the fish's ventricle, the heart could regrow fully.¹ This led to the exciting idea that if scientists could discover how the fish did it, maybe they could use the same tools to mend injuries in human hearts.

In that study, Poss et al also observed that cardiomyocyte proliferation was necessary for the regenerative process. The origin of those cardiomyocytes was unknown, however. "What we didn't know was whether those cardiomyocytes that divide are those that existed in the uninjured heart or that maybe there's a progenitor cell that creates them," Poss explains. "It's a bit of a subtle distinction."

Initial findings from the team hinted that progenitor cells might be the starting material,² but their new study in *Nature*,³ together with an accompanying report by Chris

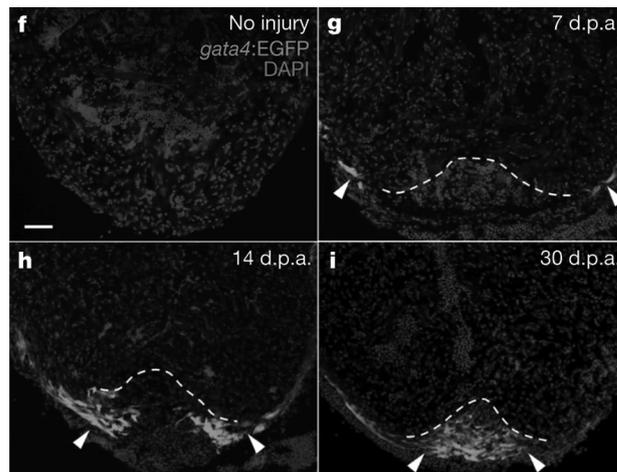


Figure. f-i, *gata4:EGFP* expression (arrowheads) in uninjured and regenerating ventricles. **Dotted line**, approximate plane of resection. Reprinted with permission from Macmillan Publishers Ltd.³

Jopling (Centre for Regenerative Medicine, Barcelona, Spain) and colleagues,⁴ confirms that this is not the case: "Our data, like theirs, say that cardiomyocytes themselves are the source tissue for at least the majority of the muscle that has regenerated back" says Poss.

In the latest study, Poss and his colleagues pinpointed cardiomyocytes as the source after showing that these cells expressed a marker of regeneration called *gata4*—a transcription factor involved in normal development of the heart. "There's not much expression of *gata4* until we injure the heart, and then, it activates," says Poss—the important point being: "We don't see it in other cardiac cell types."

Both of the teams confirmed the cardiomyocyte source by performing lineage-tracing experiments. They specifically labeled differentiated cardiomyocytes before the injury with fluorescent tags. Two weeks after injuring the heart, the tissue had regrown and it was uniformly fluorescent. "If the new cardiomyocytes came from stem cells," explains Jopling, "then they wouldn't be labeled." As the new tissue regrew, most dividing cells were concentrated around the wound and were fluorescent. This indicated that it was the cells nearest the injury site that started dividing to regenerate the injured tissue.

Kikuchi et al also showed that these regenerating cells incorporated into the existing heart tissue and reestablished normal electrical impulse conduction, which had been disrupted by the injury.

Although such an extensive regeneration process is unlike anything seen in mammals, evidence has accumulated in recent years suggesting that mammalian cardiac myocytes do retain the capacity to divide. "The notion that the mammalian heart doesn't regenerate, I think, is a

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simplistic one,” says Richard Harvey (Victor Chang Cardiac Research Institute, Darlinghurst, New South Wales, Australia). “A number of studies in mammals . . . show that cardiomyocytes can be induced to divide albeit at a fairly low level.” Just how low this level is remains debatable. Carbon dating of cardiomyocytes in human hearts has been suggested to indicate a lifetime turnover rate of 50%,⁵ whereas other estimates indicate that the entire heart is replaced several times during the course of a human life.⁶ It is also unclear whether this regeneration is provided by mature cardiomyocytes or by resident progenitor cells.⁷ Nevertheless, the ability of adult mammalian myocytes to regenerate injured tissue is limited.

Perhaps during the course of evolution, mammalian hearts have simply lost the capacity for regeneration because it wasn’t needed, suggests Jopling. After all, “Heart disease occurs later in life after we’ve reproduced.” A tantalizing finding from Jopling’s new article supports this lost-ability idea. In it, they report that when zebrafish cardiomyocytes start to divide in response to injury, they first dedifferentiate—a process that begins with disassembly of the cells’ sarcomeric structures. The interesting thing, says Jopling, is that this sarcomere disassembly is seen in mammalian and human cardiomyocytes, too. “What might be happening in mammals is that the cardiomyocytes are trying to proliferate, but for some reason, they can’t,” he goes on. “It could just be a block that we have to remove or a gene to add to give them a push to get going.”

However, it is not just that regeneration wasn’t needed in mammals, but that repair became more important, says Harvey. “The mammalian heart works at high pressure, whereas the fish heart doesn’t . . . All you need to do is put a needle through the myocardial wall and the mouse would be dead in minutes,” explains Harvey. “A repair process is appropriate in a mammal.”

So, are the zebrafish regeneration experiments, which involve chopping a large chunk out of the heart, at all relevant to humans? To address this, Poss and colleagues thought up a neat experiment in which they induced scarring in the injured zebrafish heart—a situation akin to human patients that have suffered and survived a heart attack. The team then asked what happens when regeneration is induced after repair.

“We were hoping that the scar would disappear, but what we saw instead was that the regenerative response was activated in the area and . . . would generate more muscle there.” They found that even when the repair response was stopped at the early stages of the injury to allow scar formation, restarting the regeneration process was able to clear much of the debris. If the extra muscle that was generated confers functional improvement (the team did not test this), then there is real hope that inducing regeneration could help improve scarred human hearts.

Regenerative tools are something that we might borrow from zebrafish, but there are other cardiovascular processes that we share with them and, thus, can learn about directly. In a third study published recently in *Nature*, Nicoli et al reveal the power of our little stripy friends in the study of hemodynamics.⁸

“There has been a lot done in cell culture with respect to flow and how blood flow modulates endothelial cell phenotypes, but there’s not been so much looked at in vivo,” says Nathan Lawson (University of Massachusetts Medical School, Worcester, Mass), who led the study. The transparent, rapidly developing zebrafish embryo was the perfect tool for the task. “By 30 hours postfertilization you have a complete functioning circulatory network,” explains Lawson. “You can do 12 hours of imaging and a lot of stuff happens.”

Lawson and colleagues looked at the development of the aortic arch blood vessels, which are known to remodel in response to blood flow. First, they observed how the vessels form normally, then they asked what would happen if they stopped blood flow.

Because the zebrafish embryo is so small, explains Lawson, stopping the heart doesn’t kill it. “It can obtain its oxygen directly out of the surrounding water,” he says. “Up to about day 3 of development, you can get away with not having blood flow.” The team also stopped blood flow locally using a laser to sever a blood vessel. In both cases, the lack of blood flow prevented normal growth of the aortic arch vessels.

Putting more pieces of the puzzle together, the team showed that Klf2—a transcription factor known to be responsive to blood flow—activated a microRNA called miR-126 and that, in turn, miR-126 switched on VEGF signaling. VEGF is a growth factor known to be important for angiogenesis. In mammals, miR-126 had been shown to be highly enriched in endothelial cells and to regulate VEGF signaling, vessel development, and wound repair.^{9,10} miR-126 had also been shown to be reduced in diabetic humans and mice that have deficient angiogenesis.¹¹ Thus, as far as this pathway goes, fish and mammals appear to be very similar.

“This is really one of the first genetic pathways shown to respond to flow in vivo. Very little has been done in vivo on dissecting these pathways because it’s not so easy,” says Lawson. “We hope this work will communicate to the researchers that the fish might be a great model to study how blood flow affects blood vessels.”

Lawson says there are many more questions to answer. He wants to know, for example, what are the molecules and mechanisms that are sensing blood flow at the cell surface. He thinks there may be different sensors in different parts of the vascular system and, certainly, different downstream components and responses.

The good thing is, says Lawson, “the zebrafish allows you to be creative in how you approach a problem. Whatever you can think of you can probably do.”

“When I started as a postdoc,” he goes on, “there were very few people looking at blood vessels in the zebrafish, and now, most if not all of my colleagues that use mouse, also use zebrafish.” Lawson owes his career to the little fish, he says. “I say it’s not me, it’s the model!”

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