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Growing New Limbs the Zebrafish Way

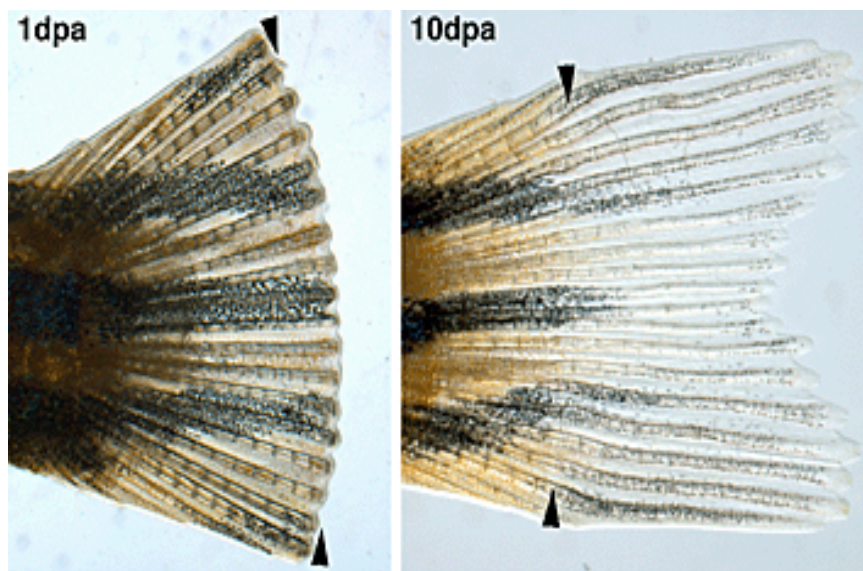


Image Title: A zebrafish tail fin one day after amputation is shown on the left. The image on the right shows that by ten days post amputation, the tail fin has regenerated. - Laboratory of Randall Moon

A lost tail fin can really slow down a zebrafish - at least for a week or so, until it grows a new one. Now scientists have shown that they can turn on or block this regeneration in zebrafish with the flip of a molecular switch. Understanding how the fish's cells coordinate the regrowth of the structurally complex fin can help scientists understand the process of regeneration, providing clues that may aid in the development of new clinical therapies, such as renewing cardiac tissue after heart disease.

The scientists said that not only will their findings advance research aimed at regenerating tissues and organs, but the discoveries could also lead to improved therapies for bone marrow transplants to restore the hematopoietic system in cancer patients.

The researchers' findings were published online December 22, 2006, as a *Development ePress* article, which is posted in advance of print publication in the journal *Development*. The senior author was Howard Hughes Medical Institute investigator Randall Moon at the Institute for Stem Cell and Regenerative Medicine of the University of Washington School of Medicine, and the joint first authors were Cristi Stoick-Cooper and Gilbert Weidinger, who designed and carried out the experiments in the Moon laboratory.

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- Randall T. Moon

Using tail fin regeneration in the zebrafish as a model system, the researchers discovered that a major cellular signaling pathway, called the Wnt/ β -catenin pathway, is central to activating the complex machinery of limb regeneration. This pathway is known to play a major role in regulating stem cells in embryonic development and adult tissue maintenance. Malfunctions in the pathway have been proven to lead to cancers, as well as being linked to bone density diseases and neurodegenerative diseases.

The Wnt/ β -catenin pathway comprises a large group of proteins that are activated when the signaling molecule Wnt binds to the pathway's cell surface receptors. This activation increases the levels of β -catenin - a master regulator of multiple genes - that reach the cell nucleus.

The researchers also found that a related Wnt protein, called wnt5b, inhibited regeneration. Wnt5b governs a signaling pathway that is independent of β -catenin. So, Wnt proteins can turn on more than one signaling pathway, and both are involved in regeneration, though with opposite roles.

"It was previously known that Wnt pathway components were expressed during regeneration, but nobody had really explored whether the pathways were indeed activated," said Moon. "And nobody had separated the two pathways and looked at their effects individually."

In their experiments, Stoick-Cooper and Weidinger genetically manipulated the Wnt/ β -catenin pathway in the fish and measured how that manipulation affected the fish's ability to regrow an amputated tail fin.

Using a fluorescent “reporter” gene that revealed Wnt/ β -catenin pathway activation by glowing like a firefly, the scientists showed that the pathway was clearly switched on during regeneration, just in the area of the body that was regenerating. Similarly, they saw that the pathway was activated during regeneration of zebrafish heart and mouse liver, which they said suggests that the pathway may function in regeneration across species. They further noted that the Wnt/ β -catenin pathway was activated during the formation of the undifferentiated cells that proliferate to regenerate the tail fin.

They next genetically engineered fish in which they could switch off the Wnt/ β -catenin pathway by exposing the fish to warm water. Doing so, they found, completely blocked regeneration. They could also accelerate regeneration by enhancing Wnt/ β -catenin signaling. The researchers noted that this is a significant finding for the new field of regenerative medicine, in which a means of enhancing regeneration is an invaluable tool.

In contrast, when they engineered fish to activate the *wnt5b* gene in response to warm water, regeneration was inhibited. The opposite is true in fish with a mutation in the *wnt5b* gene, in which they found that regeneration was augmented. This is what one would expect, Moon noted, since the loss of a functional inhibitor is a double negative -- meaning that regeneration should be accelerated.

“These experiments showed that there is a completely novel and unexpected mechanism that antagonizes the regenerative process,” said Moon. “There had been other studies indicating that *wnt5b*-like genes could block the Wnt/ β -catenin pathway, but no one had examined whether this antagonism occurs in the context of the normal regenerative process.”

“Cristi and Gilbert's experiments rigorously establish through genetic approaches that the Wnt pathways are functionally important in regeneration,” he said. “More generally, they show that in studying injury or inflammation in any context, investigators should explore whether Wnt signaling is involved. These experiments suggest that Wnt signaling is a universal component of regenerative pathways in animals,” he said.

According to Moon, the findings by Stoick-Cooper and Weidinger will have clinical implications for tissue regeneration, as well as for encouraging growth of stem cells. Stem cells are immature, undifferentiated cells that are capable of maturing into a variety of mature cell types.

“Wnt/ β -catenin signaling plays an important positive role in the differentiation of stem cells and progenitor cells that are required for regeneration,” he said. “It agrees with previous studies in which our laboratory showed in animals that activating this pathway increases the success of transplants of blood-forming hematopoietic stem cells. Such transplants in cancer patients whose immune systems have been destroyed by radiation or chemotherapy are invaluable as therapy; and sometimes they fail

because they transplanted cells do not engraft into the bone marrow. We believe that enhancing the Wnt/ β -catenin pathway will increase the success rate of such hematopoietic stem cell transplants,” he said.

In other clinically-related studies, Moon and his colleagues are exploring whether activating the Wnt/ β -catenin pathway can enhance differentiation of human embryonic stem cells into cardiac cells that could be used to treat heart disease

Moon and his colleagues are also exploring how the myriad different kinds of cells involved in regeneration respond to Wnt-activating signals and, in collaboration with HHMI investigator Leonard I. Zon at Children's Hospital, Boston, how injury switches on Wnt/ β -catenin signaling. The researchers are optimistic that Wnt signaling will be an important therapeutic target in the growing field of regenerative medicine.