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Regenerating Worms Help Elucidate Stem Cell Biology

Using a tiny flatworm best known for its extraordinary ability to regenerate lost tissue, researchers have identified a gene that controls the ability of stem cells to differentiate into specialized cells. The gene encodes a protein that is most similar to the protein PIWI, an important regulator of stem cells in organisms ranging from plants to humans.

The replacement of tissue lost to injury or shed during the body's normal activities is essential for the survival of most organisms. The new study, published in the November 25, 2005, issue of the journal *Science*, helps scientists understand how stem cells make this process possible. The research, performed at the University of Utah School of Medicine, was carried out by Helen Hay Whitney postdoctoral fellow Peter W. Reddien (now an Associate Member at the Whitehead Institute for Biomedical Research), and led by Howard Hughes Medical Institute investigator Alejandro Sánchez Alvarado.

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Salamanders, zebrafish, and other organisms are capable of regenerating entirely new body parts. Although the human body does not face such demands, it is constantly replacing lost cells. For example, blood replenishes itself, wounds heal, and the lining of the gut sloughs off and is restored. Nowhere, however, is the process of regeneration more dramatic than in the freshwater flatworm planaria. Cut one of these animals in half, and a week later, two fully functional worms will have developed from the pieces. Cut a piece that is $1/279^{\text{th}}$ the size of the animal, and it too will regrow into a complete worm.

The process, scientists know, is dependent on stem cells in the adult planaria known as neoblasts. Like all stem cells, neoblasts have the capability to develop into a variety of different cell types, meaning they can transform

themselves into whatever tissue is needed after injury, be it intestine, skin, or brain. Even in the absence of injury, these cells are critical to maintain a healthy worm, as they are also responsible for replacing tissue that has been lost naturally. Scientists are just beginning to explore the molecular mechanisms that control adult stem cells, so, said Sánchez Alvarado, it's too soon to know how similar these mechanisms are in planaria neoblasts and other organisms' stem cells. "But at least at the gross morphological level and gross biological functions, they compare quite well," he said.

Destruction of a planarian's neoblasts, which occurs when scientists expose the animal to radiation in the laboratory, is devastating. "The animal will survive on the virtue of its differentiated cells," Sánchez Alvarado said, "but as the tissues begin to turn over and there are no stem cells to replace such tissues, the animal begins, basically, to fall apart." It degenerates in a very specific way, he explained, with the tip of the head beginning to regress, followed by a curling up of the sides of the body. Not surprisingly, worms without neoblasts also lose their ability to regenerate.

With their unparalleled capacity for regeneration and the many environmental cues that influence the division and differentiation of their neoblasts, Sánchez Alvarado considers planaria an excellent model to tease out the intricacies of adult stem cell biology. "I think they probably have a lot to teach us about how a population of stem cells is being regulated *in vivo*, rather than in a Petri dish," he said. So Sánchez Alvarado and his colleagues set out to understand exactly how neoblasts carry out the remarkable maintenance and recreation of the varied tissues that make up a flatworm.

Earlier this year, they got their first clues when they individually turned off 1,065 of the worm's genes, and found 240 that were involved in some aspect of regeneration. Importantly, Sánchez Alvarado noted, 85 percent of these genes are found in the genomes of other organisms, including humans. In the current study, the scientists zeroed in on one of these genes, called *smedwi-2*, that was active in dividing neoblasts.

Smedwi-2 belongs to the Argonaute/PIWI protein family and is most similar to PIWI proteins found in fruit flies. According to Sánchez Alvarado, PIWI proteins have been shown to play a role in regulating stem cells in plants and fruit flies, as well as humans. "This encompasses millions of years of evolution," he said. "Still, we don't know exactly how this particular gene is doing its function in any of these organisms."

To find out, the scientists used a technique known as RNA interference to specifically turn off the *piwi* gene in planaria. When they did this, they found that worms had the same defects as those whose neoblasts have been destroyed by radiation—head regression, curling, and the inability to regenerate—suggesting that the gene was needed for normal neoblast function.

The researchers examined *piwi's* role more closely, and found that when they amputated part of a worm where the gene had been turned off, the stem cells were still able to detect the wound. Amputation triggered the stem cells to

divide, as in normal worms, and the daughter cells traveled proficiently to the part of the body where they were needed. However, once they arrived, they failed to replace the lost tissue.

When neoblasts divide, they produce at least two cell types - one copy of the original, and one cell that can develop into a specialized cell to replace a lost cell elsewhere in the body. The researchers found that without *piwi*, the daughter cells from this division failed to differentiate into a specialized cell once they'd reached their destination. Based on their findings, Sánchez Alvarado said, "We think that *piwi* is actually involved in producing daughter cells that are competent to restore aged differentiated cells during homeostasis as well as missing tissues after amputation. Unlike what's been thought about *piwi* for some time, which is that it was required to maintain the stem cell, we think that's not happening here. The stem cells are being maintained by another mechanism, and it's the division progeny, instead, that is being affected."

There's some evidence, Sánchez Alvarado said, that *piwi* plays a similar role in regulating the progeny of adult stem cells in humans. He cautioned that more work is needed to determine just how functionally similar the factors regulating stem cells in planaria are to those in higher organisms. But so far there's good evidence that many of the important genes are the same, he said. And the current study begins the detailed analysis that will be needed to establish whether this humble worm can illuminate the mechanisms underlying the unique biology of stem cells.