Growth Industry

A STRANGE-LOOKING SALAMANDER PROVIDES A WINDOW INTO HOW VERTEBRATES REPAIR THEMSELVES

By John Pastor

With flaring red gills that jut out of a milky white body, and round, black eyes that never blink, the axolotl salamander has been trolling tropical pools for 300 million years.

But only in recent years have scientists begun to appreciate the axolotl's amazing ability to repair injuries that would leave humans and other mammals paralyzed — or worse.

"The axolotl is the champion of vertebrate regeneration, with the ability to replace whole limbs and even parts of its central nervous system," says Edward Scott, a professor of molecular genetics and microbiology in the UF College of Medicine and director of the McKnight Brain Institute's Program in Stem Cell Biology and Regenerative Medicine.

While worms, starfish and other invertebrates can perform wondrous feats of regeneration — some starfish can completely regenerate from a single, remaining arm — the axolotl is a vertebrate, with hind limbs and forelimbs — like us. That makes them the closest relative to humans that can regenerate spinal cords, limbs, internal organs and substantial amounts of brain.

The question is why axolotls regenerate so well, while people, by comparison, do not.

The answer is important enough for the National Institutes of Health to invest $2.4 million in a Grand Opportunity grant to Scott and co-investigator Dennis Steindler, executive director of the McKnight Brain Institute. The grant is funded through the American Recovery and Reinvestment Act of 2009.

Working in association with the UF-led Regeneration Project, an international collaboration of life scientists, their objective is to create the genomic tools necessary to compare the extraordinary regenerative abilities of the axolotl with established mouse models of spinal cord injury, stroke, traumatic brain injury and other neural conditions.

One way to do this is to "turn off" the creatures' regenerative abilities, then see if they can be turned back on again.

At the MBI's Stereotactic Radiosurgery Laboratory, three axolotls hover serenely in a water-filled plastic box, only the gentle flutter of their blood-red gills betraying life.

Scott places one of the salamanders in the sights of a linear accelerator, which will deliver precise doses of radiation to the animal's liver and spleen, two organs that seem to be the source of its blood stem cells.

"What we want to accomplish here is something like an axolotl bone marrow transplant," Scott says.

Ray Carson

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The animals' opaque, white body comes in handy. A bright light reveals their internal organs. No sophisticated imaging necessary.

"They're ugly, but in a cute way," Scott says. "You can practically see through them, even when they are adults, and it helps us to do some amazing things."

The idea is to disrupt the axolotl's regenerative ability and then see if it can be restored with transplanted stem cells. Researchers will be able to follow the origin, movement and destination of the cells using green fluorescent proteins. When produced by genetically modified cells, these proteins have the useful quality of glowing vivid green under ultraviolet light.

"Axolotl stem cells look very different than human ones and are concentrated in different areas of the body," Scott says. "You can flush the bone of the axolotyl and get maybe 50 cells. However, the liver and spleen are full of them, which mirrors the embryonic stages of humans and mice. Hopefully we will track stem cell migration from fetal liver to bone marrow through this process."

This experiment is but a single step toward determining whether the axolotyl's regenerative talent is dormant within humans, ready to be revived with new drugs, reagents or treatments. Axolotls and humans share about 90 percent of their genes, and the team has already referenced human and mouse genes with axolotl counterparts.

"Ultimately, what makes the axolotl a great model for regeneration is that the model systems we are most familiar with — mice and humans — do not regenerate very well," Scott says. "By comparing how a mammal and a salamander respond to injuries, we can identify genes or proteins that we can now add back to the mammalian system to make it regenerate better."

The issue of what controls organ regeneration was named among the top 25 major questions facing scientists

in the next quarter century by *Science* magazine in 2005. With medical science adding years to the human lifespan, the importance of rebuilding and restoring old tissue and organs is growing. But science had to enter the 21st century to begin to take advantage of the highly regenerative axolotl as a model for human disease.

"Only now have new genetic, molecular and cellular technologies, as well as scientific knowledge of the salamander, mouse and human genomes and 'regeneromes,' risen to a level where scientists can compare systemwide responses to injury," Steindler says. "I am extremely hopeful with the discoveries being made in comparative regenerative biology that the questions surrounding cell and tissue regeneration in the human following injury or disease are going to be answered."

Steindler founded the Regeneration Project in 2007 as an international effort to overcome barriers that have limited progress in regenerative biology and medicine. Featuring annual think tank-style meetings of stem cell and developmental biologists, biomedical engineers, genomic researchers and clinicians, the initiative promotes information sharing across disparate scientific fields.

It would be unlikely at most symposia, but quite reasonable at a Regeneration Project meeting, for a molecular cell biologist from the Max Planck Institute in Germany, a urological surgeon from Wake Forest University in North Carolina, a tissue engineer from the University of Pittsburgh and a stem cell biologist from the University of Florida to engage in a conversation about their research.

The glue that holds these diverse interests together are research fellows, another Regeneration Project innovation. Introduced as the "experiment within the experiment" by Steindler at the first project meeting, the fellows enhance idea sharing and conduct joint experiments to find answers in the biological systems of simple animals that can be applied to the more complex tissue reconstruction needed in humans.

"It is going to take broad, multidisciplinary collaborations across a number of scientific fields to improve health care, but we are making that happen," Steindler says. "I think the Grand Opportunity (GO) grant shows that these efforts are recognized and valued on a national level."

"We are bringing together the best of the developmental biology world with the best of the stem cell world and starting the conversation, with the focus on how to get regeneration to work in a mammal," adds Scott. "Essentially, our body can heal itself, and that's why many of us live to be 80. But we can't do things like grow an arm or finger as we did in the early stages of our development. We want to learn how to turn those systems back on in people."

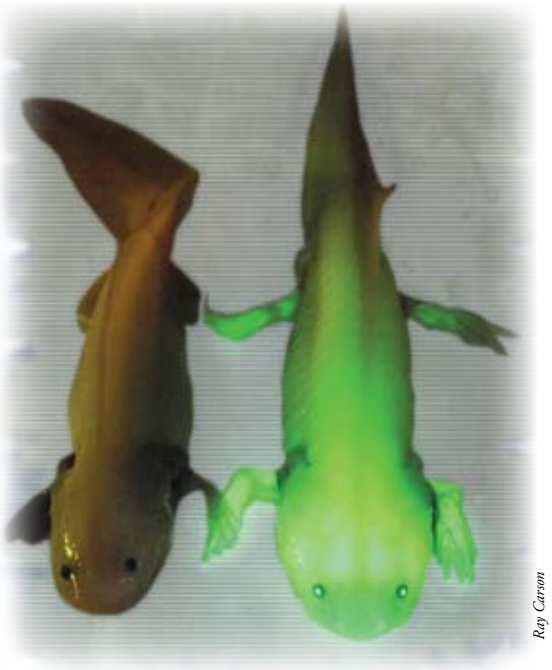
GO grants are intended to support research that lays the groundwork for whole new fields of investigation with the promise of advancing biomedical research and improving health care.

"This important model of regeneration is one of several being developed in organisms that can repair themselves, using genetics to find links to mammals," says Naomi Kleitman, repair and plasticity program director at the National Institute of Neurological Disorders and Stroke. "We'll continue to watch the progress of these exciting studies to ensure that discoveries of genes that promote regeneration are one day applied to improving human health."

The Regeneration Project is also supported by the Thomas H. Maren Foundation and the Jon L. and Beverly A. Thompson Research Endowment, the UF Office of the Vice President for Research, and an anonymous donor.

Maren, a founder of the UF College of Medicine who died in 1999, was a staunch advocate of using comparative organisms to solve human health problems. His studies of marine life led him to understand the chemistry and biology of an enzyme called carbonic anhydrase, which influences the production and flow of fluid in the eyes, brain, spinal cord and lymph system.

The result was the development of dorzolamide, a top-selling drug for glaucoma that goes by the trade name Trusopt. Royalties from Trusopt support the Maren Foundation and Maren's example helped inspire the Regeneration Project, Steindler says.



Ray Carson

Green fluorescent protein allows scientists to track where cells travel in the axolotl's body.

Ray Carson

David Blankenship

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"Dr. Maren's wife, Emily, told me her husband valued the strategy of selecting the right organism to answer scientific and medical questions, and that he was familiar with axolotl studies," Steindler says. "He invented Trusopt because of comparative biology, looking at sharks and other marine animals. In that same way, we want to arrive at a technique that can regenerate spinal cord tissue and other tissues of the body."

An authority in adult human brain stem cell biology, Steindler is exploring the potential of what are known as multipotent astrocytic progenitor cells to treat disease and spinal cord injury in humans through their ability to transform into a limited number of needed cell types, such as neurons — the brain's workhorse cells.

Axolotls apparently have the same sort of cells in their brains. The difference is the axolotl cells can repair a vast amount of damage in comparison with their human cousins. Malcolm Maden, a professor of biology at UF and a Regeneration Project member, has shown that axolotls can regenerate up to a third of their brain matter in six weeks, reproducing a structurally sound, functional brain.

Maden has also found hope for recreating some of the axolotl's regenerative capacity in humans through his research on the salamander's limbs.

In a paper last year in the journal *Nature*, Maden and six colleagues found that cells from the salamander's different tissues retain the "memory" of those tissues when they regenerate.

Standard mammal stem cells operate the same way, albeit with far less dramatic results. They can heal wounds or knit bone together, but not regenerate a limb or rebuild a spinal cord. What's exciting about Maden's findings is they suggest that harnessing the salamander's regenerative wonders is at least within the realm of possibility for human medical science.

"I think it's more mammal-like than was ever expected," says Maden. "It gives you more hope for being able to someday regenerate individual tissues in people."

Also, the salamanders heal perfectly, without any scars whatsoever, another ability people would like to learn how to mimic, Maden says.

"If we can figure out why the axolotl does this better than the human and mouse," says Steindler, who has begun cell transplant studies using axolotls and mice, "then we are really going to get somewhere in human regeneration."

Ray Carson

As discoveries are made, more researchers will want to use the axolotl as a model for exploring regenerative techniques, which in turn will cause more grant applications to the NIH for axolotl research. In that event, UF and other Regeneration Project scientists will be well positioned to apply for the funding.

In the short term, regeneration scientists are working to elevate the axolotl to the level of scientific mainstays such as roundworms known as *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and, the king of the model organisms — the mouse — as a tool to study biological processes and human disease and injury.

Arlene Chiu, a scientific adviser for the Regeneration Project and director of New Research Initiatives at Beckman Research Institute of the City of Hope in San Francisco, thinks axolotl research could increasingly appeal to the NIH because it touches on many of the institute's priorities, including application of genomics, development of treatments,

invigoration of the biomedical research community and global health.

Besides that, the model has something special.

"The axolotl is the highest, most complex organism that can still do this clever trick of completely reconstructing a whole body part in adulthood," Chiu says. "I like to think of it in construction terms where we need both the materials such as bricks and beams and the architect's plans. In regenerative medicine, can we learn where the biological blueprint resides, and understand the basis of restoring and reorganizing many different types of lost cells and tissues? Muscles, bones, nerves and blood vessels all have to be reconstructed at the right time and in the right place, all in perfect coordination with the original biological master plan.

"It may sound like science fiction, but the reality is the salamander is able to do all of these things," she says. "We are not so far removed that we can't relate to them, learn from them and try to apply their secrets to improve our capacity to regenerate."

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