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*MASTER OF  
REGENERATION*



*by Kendall Powell  
illustration by Jason Holley*

*Once a high school biology oddity, planarians are moving into the spotlight to reveal secrets of self-renewal.*

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As the plane touched down in Barcelona on a Saturday afternoon in September 1998, Alejandro Sánchez Alvarado was excited to see the runway pavement slick from a recent rainfall. Rain would have filled up the abandoned fountains in Parc de Montjuïc where he and Phil Newmark hoped to trap their quarry. »

# THE TWO HURRIED IN A CAB TO THE NEAREST BUTCHER FOR A SLAB OF SHEEP'S LIVER,

their target's preferred dinner, and then went straight to their hotel room to fashion traps by stuffing the liver bait into empty bottles. They needed to set the traps in half a dozen of the park's fountains before nightfall. They had only a long weekend and they were taking a big gamble that their traps would lure swarms of what they hoped would be developmental biology's next important model organism: the planarian flatworm, *Schmidtea mediterranea*.

Transforming the planarian worm from a fountain-dwelling, biology classroom novelty—cut one in quarters, get four worms—to a workhorse of molecular developmental biology was an adventure both inside and outside the laboratory. The quest took Newmark and Sánchez Alvarado—and later Peter Reddien—to art stores, to amputation assembly lines, and into the genome that controls one of the most robust abilities to regrow body parts anywhere in the animal kingdom.

All three men—Sánchez Alvarado and Newmark are now HHMI investigators, Reddien is an HHMI early career scientist—arrived at the same brilliant idea: if we want to understand how regeneration

works, shouldn't we be using the master of regeneration, the planarian? It's a simple flatworm with rudimentary eyes, brain, gut, and gonads but no circulatory or respiratory systems. A fraction of the worm, 1/279th or just 0.3 percent, can regenerate a whole new animal. Cut off its head, a new one grows back within 10 days.

Aside from its capacity to self-renew, the planarian is a beguiling creature with its googley-eyed cartoonish look contrasted with a gliding swimming elegance. But when these researchers started, the animal had only a handful of identified genes. How to grow them optimally in the laboratory even needed work.

## REGENERATION REVIVAL

The scientists had all worked their way through the literature back to Thomas Hunt Morgan, who studied regeneration in planarians around the turn of the 20th century. Before he became the father of modern genetics and championed the fruit fly *Drosophila melanogaster* as a model organism, Morgan made a rich set of observations about the planarian's ability to regrow itself. Through meticulous cutting and weighing, he calculated the 1/279th fraction. He also noted that if he made large cuts to amputate the head and the tail of an animal, leaving only a very thin middle section, he sometimes got a two-headed animal in its place.

Work on planarians petered out in the 1960s and 1970s as organisms more user-friendly for genetic and molecular tweaking, like the fruit fly and the roundworm *Caenorhabditis elegans*, took center stage.

In 1995, Sánchez Alvarado became a staff associate in the department of embryology at the Carnegie Institution for Science in Baltimore, Maryland. He made it his mission to find the best organism to study regeneration in an adult animal.

The conventional thinking at the time was that regeneration was simply a recapitulation of embryonic development pathways.

That logic never sat right with Sánchez Alvarado.

"If animals are just redoing embryological developmental events, then everyone should do it. And we wouldn't care as much about having health insurance plans," he says. "Instead, you are asking an adult animal to form a *de novo* structure within an adult context."

After reading Morgan's 1901 book *Regeneration*, Sánchez Alvarado was convinced that regeneration was actually a broad phenomenon in the animal kingdom, not just an evolutionary quirk relegated to a handful of strange animals. Happy to get lost in the stacks of the Library of Congress, Sánchez Alvarado marched through the literature and found examples of regeneration in every animal phylum, from the ancient Cnidarian hydra right up to the Chordata, or vertebrate, salamander. More surprising to him were the examples in all the phyla in between.

"Almost every phylum has an example of an animal that can regenerate tissues when faced with injury and amputation," says Sánchez Alvarado, now at the University of Utah. Even humans, after all, can regenerate significant portions of organs such as skin and liver. "Maybe the thing to do was to identify an invertebrate in which you could test hypotheses rapidly and begin ruling out what is and is not happening in regeneration," he says. "Planarians really fit the bill."

## A CHANCE MEETING

Amid antique locomotive engines at the National Railway Museum in York, England, Sánchez Alvarado sat next to Newmark at the 1996 annual meeting banquet of the British Society for Devel-

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*Below: Alejandro Sánchez Alvarado says that all model organisms are chosen because they exaggerate a particular biology.*

opmental Biology. At the time, Newmark was in Jaume Baguña's lab at University of Barcelona as a Damon Runyon Cancer Research Foundation postdoctoral fellow, learning everything he could about fundamental planarian biology to eventually set up a line of research in the United States.

"Here I found another member of my tribe," recalls Sánchez Alvarado. The two contemporaries, just one year apart in age, hatched a plan to convince the Carnegie Institution and Damon Runyon to let Newmark be a postdoc with Sánchez Alvarado. The two would attempt to turn planarians into a cultured lab animal that could serve as a system for deconstructing the molecular pathways that control regeneration.

Both Carnegie and Damon Runyon "really took a risk and supported us," says

Newmark. "There were plenty of people [along the way] who thought this was craziness."

The two personified dogged persistence. Early on, they spent a sleepless weekend cutting heads and tails off of about 1,000 animals. These experiments would tell them in the coming months which genes were expressed in intact heads and tails compared with regenerating heads and tails. Newmark also worked on modifying an *in situ* hybridization protocol, which is used to mark specific genes with a dye to see where the gene is turned on in the whole animal. It wasn't easy. The animals' mucus covering tended to suck up the dye and turn everything dark blue.

With almost any method they attempted for tracking the planarians'

stem cells—which are called neoblasts and make up roughly 30 percent of the animal's cells—the team had to work through trial and error. They figured out how to label the neoblasts and began making libraries of the genes expressed during various stages of regeneration.

During his time at Carnegie, Newmark also created a genetically identical line of worms by cutting and growing up pieces from one individual collected from the Barcelona park. That line has been going for almost 12 years and is used by all the planarian laboratories in the United States. No more mucking through dormant fountains.

In 1998, Andrew Fire, working downstairs from the two at Carnegie, had discovered a way to silence specific genes in *C. elegans*. The method, called RNA



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*Below: Along with Sánchez Alvarado, Phil Newmark (left) and Peter Reddien brought planarians into the molecular era.*

interference, or RNAi, works by introducing a double-stranded RNA that matches the message of the gene a researcher wants to knock down. The RNAi sets off a process in the cell that destroys the mRNA message. The method, for which Fire shared the 2006 Nobel Prize in Physiology or Medicine with HHMI investigator Craig Mello, effectively turns off the gene.

Fire asked Newmark and Sánchez Alvarado to try RNAi in planarians to see if the method would work beyond *C. elegans*. Sánchez Alvarado first tried silencing the planarian tubulin and myosin genes since the resulting proteins are found in every cell and he had the dyes to track them. He recalls looking at the treated worms under the microscope at 1:00 or 2:00 a.m.: their regeneration stumps, or blastemas, had no tubulin or myosin.

Now they had a loss-of-function assay for the planarians—a tool with which to ask, if this gene is missing, how does it affect the regeneration mechanism? All they needed was some regeneration-specific genes to test.

“We needed to clone genes like there was no tomorrow so that we could do RNAi screening. That kept us occupied for eight years or so,” says Sánchez Alvarado. Newmark calls the RNAi work a crucial breakthrough from their years at Carnegie: 1997 to 2001.

“Now we could do functional biology. We could ask whether inhibiting the expression of a given gene could disrupt the regenerative process. We could really learn something from these animals. It was a fantastic time,” says Newmark, now at the University of Illinois at Urbana-Champaign.

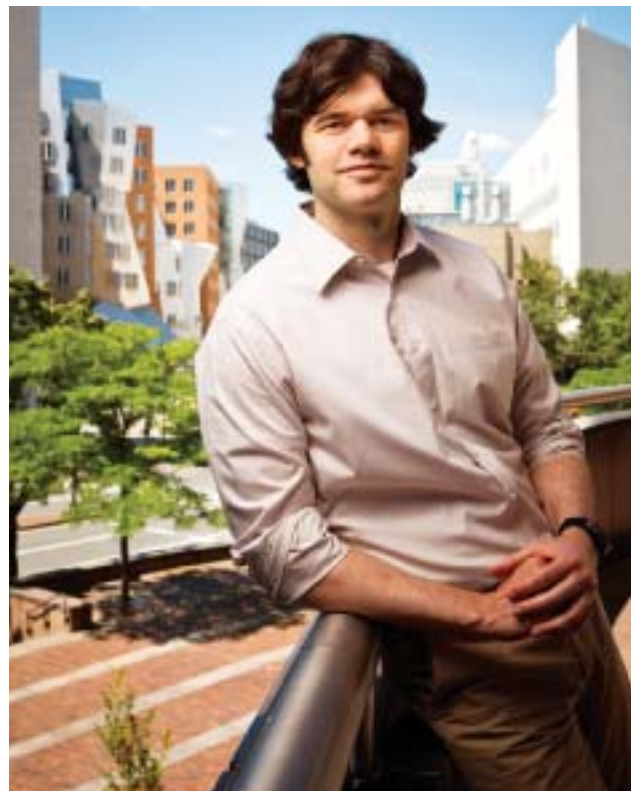
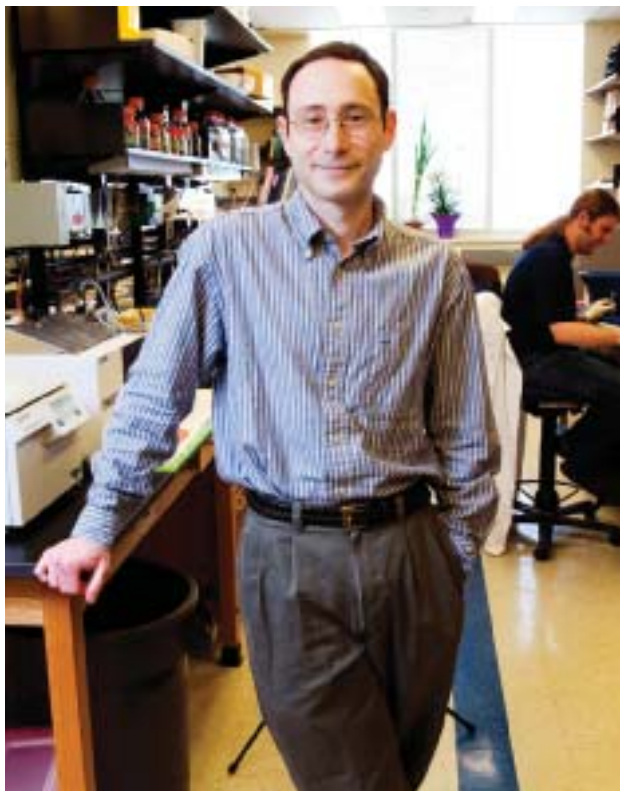
### LEARNING CURVE

Reddien discovered Morgan’s work during his graduate studies. That quickly led him to Sánchez Alvarado and Newmark’s 1999 *Proceedings of the National Academy of Sciences* paper on the RNAi work.

“The things they had shown gave me hope that turning planarians into a molecular model organism would work,” Reddien recalls. “They were bold with their choices and had taken a massive risk.”

Reddien joined Sánchez Alvarado as a postdoc at the Utah laboratory and his first task was to use RNAi for a large screen of planarian genes. It was still unclear if this approach could silence genes well enough to show phenotypes—that is, to show physical or behavioral defects, such as improper regeneration in a worm.

He first chose to test 30 genes from a list of nearly 4,000 genes cloned and sequenced



Newmark: Darrell Hoemann / AP, ©HHMI Reddien: Jason Grow

at Carnegie but was disappointed to see no defects in the first trial. He speculated that perhaps the worms had to go through two rounds of regeneration before defects would appear. That did the trick.

“I started seeing phenotypes,” he says. “Animals would fail to regenerate, or were paralyzed, or had weird lesions all over their bodies. It was very dramatic and very exciting.” So much so, the entire lab headed to Squatters brew pub in Salt Lake City for a celebratory Champagne toast.

Not all of Reddien’s postdoc time went so swimmingly, however. Working with a worm virtually unknown in molecular circles and handled by only a few labs around the world had its headaches.

Reddien played with tricking the worms into gobbling up the bacteria that produced the double-stranded RNA. He also made trips to an art supply store to find the best materials, like heavy black paper, for taking photographs of the semitranslucent worms through the microscope.

“That was part of the fun and adventure for me, but it was also very challenging,” says Reddien, now at the Massachusetts Institute of Technology’s Whitehead Institute for Biomedical Research. “There is no protocol book that you can just pull out and implement. We have to start from scratch each time we would like to use a new method.”

Reddien eventually optimized the RNAi to do a large-scale screen to knock down 1,065 planarian genes and search for defects. Reddien and two lab mates made more than 53,000 amputations to worms with a razor blade during the screen. They found 240 genes that when silenced produced some kind of defect; 85 percent of these genes are conserved in other animals, including humans.

As a model organism, the goofy-looking flatworm had arrived at a critical juncture.

## *THIS IS THE TYPE OF SCIENCE YOU DREAM ABOUT AS A KID. WE ARE STUDYING PROCESSES THAT ARE DRAMATIC AND BROADLY IMPORTANT.* —Peter Reddien

“I was getting the first collection of planarian phenotypes the world had ever seen,” recalls Reddien. The work was published in *Developmental Cell* in 2005.

With the molecular tools in hand to light up, track, and silence the planarian’s stem cell genes, as well as much of its genome completed (a project also spearheaded by these three), the scientists could begin to map genes onto the key stages of regeneration. They could move on to the tasks that really charged them up: finding genes that give the neoblast cells their stem cell identity, genes that direct them to respond to a wound, genes that help form the regeneration stump, or blastema, and—most intriguing of all—genes that tell the animal which body part is missing.

### *EYE-CATCHING MODEL*

These questions will occupy the three labs (and any others that crop up from their descendants) for the next decade. Newmark’s group has set out to understand how neoblasts become other kinds of cells, particularly germline cells. Planarians can resorb their whole reproductive tract in times of starvation and then regrow ovaries, testes, sperm, and eggs when food is plentiful again. They also regenerate germ cells after amputation.

His lab group has identified genes that block germ cell differentiation and a

subset of these genes are specific to flatworms. Newmark jumped on that subset, because it opens up an opportunity to understand a neglected tropical disease.

Schistosomiasis affects 200 million people worldwide who come in contact with waters infested by a close planarian relative, the schistosome. The female worms lay eggs in an infected human, causing inflammatory reactions that can damage the liver and intestines and cause malnutrition and learning difficulties in children. Newmark’s findings could lead to a way to disrupt egg production in schistosomes.

“It’s fascinating biology and we’re in a good position to contribute to understanding a very important disease that’s not getting much attention,” he says.

Reddien’s group continues to pursue genes he identified in the RNAi screen that gave some of the most interesting regeneration defects. In a follow-up screen, he and his postdoctoral fellow Chris Petersen discovered the most eye-catching worm to date.

“Chris came across the two-headed phenotype. When I looked through the microscope I just about fell out of my chair. We knew we had something very important,” says Reddien.

Morgan had observed the two-headed worm, which plays a perpetual game of  
*(continued on page 48)*

part in a research project, he says, requiring approval by an ethics panel and village elders as well as cooperation with traditional healers. “You have to respect the customs and the organization of the community. You have to find a way to explain what you plan to do, what risks are involved, and the likely benefits for the community and perhaps for mankind in general.”

In one project, Djimdé’s group, in collaboration with Wellem’s NIAID lab, is examining the parasite’s resistance to quinine, the time-tested drug derived from tree bark that is often used to treat the most severe cases of malaria. (Quinine is a natural product that differs markedly from chloroquine, which was synthesized by German scientists and refined as an antimalarial drug by U.S. researchers during World War II.) He is also trying to determine the safety of, and whether resistance is emerging to, the newest generation of ACTs.

The fight against malaria has continued for centuries—and intensified in recent years with the advent of the World Health Organization Global Malaria Programme and related efforts sponsored by major aid organizations. In recent years, optimistic funding agencies, such as the Bill & Melinda Gates Foundation,

have suggested that malaria can be eliminated, and more than two dozen countries have launched efforts that they hope will stamp out the disease, with some progress reported.

Plowe and Djimdé support the ambitious goal. “The malaria research world has been transformed by the call for eradication,” Plowe says. But the two say it will take years of research to stop malaria in nations where the disease is now endemic and widespread.

“I think eradication is possible, but not in the near future,” says Djimdé. “The tools we have today—notably, the artemisinin-based combination therapies and mosquito nets, with the possibility of an efficacious vaccine against malaria—can be very effective if they are available and are deployed. In specific communities, they can lessen malaria to the extent that it is no longer a serious public health problem.

“I don’t think we have the tools to [eradicate malaria] yet. This will require sustained funding and decades of research,” he adds.

Both Djimdé and Plowe will continue to lead the way. “Djimdé is now one of the best-known researchers in all of Africa,” says Plowe. Although they’re using different approaches to tackle malaria, the two scientists remain friends after nearly 20 years—sometimes mentoring the same students and discussing their separate projects. Even their families are close. “It’s been a great friendship.” ■

tug-of-war with itself, a century earlier. But Petersen and Reddien were in a better position; they knew which silenced gene had caused the oddity. It was beta-catenin, a key molecule in the Wnt signaling pathway that controls body plan polarity in many animals during embryonic development. Beta-catenin transmits the Wnt signal into the cell’s nucleus, where it directs changes to a set of other genes’ expression levels.

The two showed that, normally, beta-catenin turns on genes that suppress head formation and promote tail formation. However, when beta-catenin is absent, the default is to produce a head at any wound site, according to their 2008 *Science* paper. At the same time and also published in *Science* in 2008, Sánchez Alvarado’s group created an animal with boosted levels of beta-catenin; when its head was amputated, a tail grew back instead.

“This is the type of science you dream about as a kid. We are studying processes that are dramatic and broadly important,”

says Reddien. He says this study captivates him because it hammers home the idea that choices are being made at the sites of wounds. He wants to uncover how those decisions happen.

“We could not ask these questions in *Drosophila* or *C. elegans*. Planarians’ biology is very different and they enable a whole suite of questions that couldn’t be addressed in existing model systems that do not regenerate robustly,” notes Reddien.

Brenton Graveley, one of a handful of scientists starting to use planarians in biomedical research, gives Sánchez Alvarado, Newmark, and Reddien all the credit. “The three of them brought planarians into the molecular era,” says Graveley, a molecular biologist at University of Connecticut Health Center in Farmington.

The early genome work by Sánchez Alvarado and Newmark that showed planarian genes were closely related to human genes “propelled planarians to the forefront,” he adds. “If you are not going to work on human or mouse cells, then the leap in

translating what you find in planarians to actual human stem cell function is going to be much shorter,” he notes.

Sánchez Alvarado speaks fondly of his quirky, but potentially powerful, pet organism: “What model system isn’t funky? *C. elegans* has such a defined cell lineage, it’s uncanny. Morgan chose *Drosophila* because they were such prodigious egg layers. All model organisms got chosen because they exaggerate a particular biology.”

Sánchez Alvarado remembers the flight home after the fountain-foraging expedition in Barcelona. He and Newmark camouflaged the cooler of live worms with cardboard and duct tape. “It looked so suspicious, but they let us through.”

The journey into planarian self-renewal has been both adventurous and arduous. “It was hard,” he admits. “But when you look at the animal, and the wild type is already unbelievable—I mean, the guillotine would not work on this guy—I thought, ‘If we can actually go in and perturb these things, how amazing it would be.’” ■



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