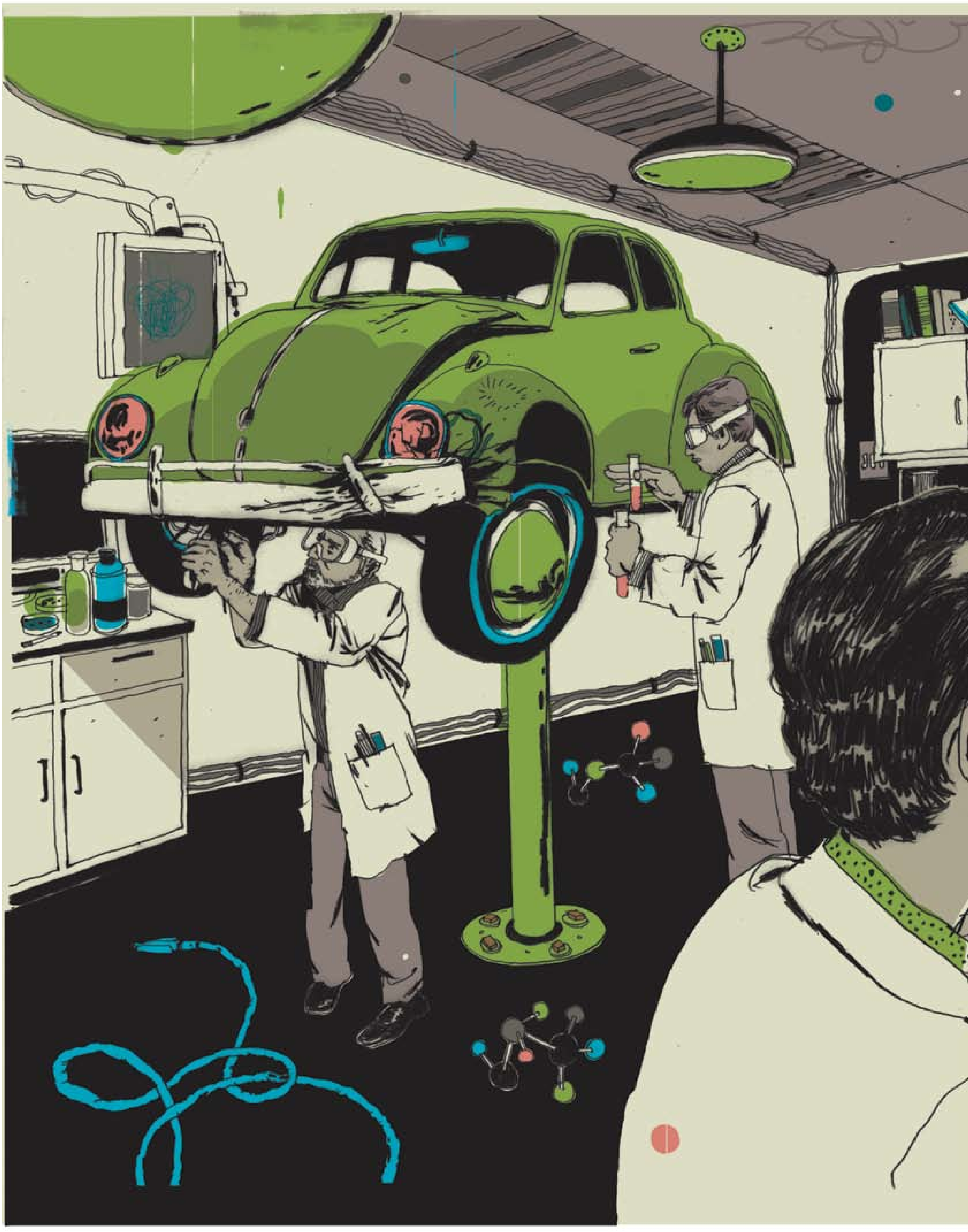

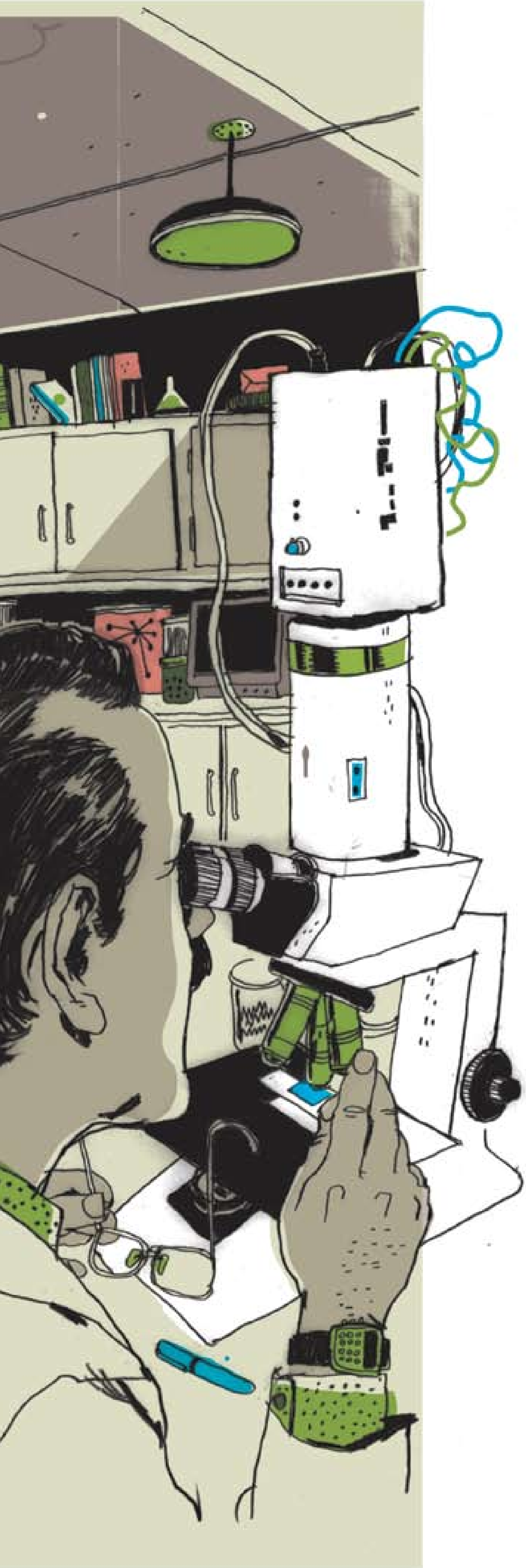

Regeneration for Repair's Sake

If a salamander can regrow a lost limb, why can't we? Or should we be aiming for a different goal?

by Kathryn Brown

illustration by Josh Cochran

The two-headed tadpoles were a shock.

When he unexpectedly bred a dish of the aberrant creatures 19 years ago, Randall T. Moon soon realized he was witnessing the power of regeneration—in the form of a protein that helps regulate development.

"We knew this protein, called Wnt, helped flies develop, but its role in vertebrate models was completely unknown," says Moon, now an HHMI investigator at the University of Washington. The memorable tadpoles had higher than normal amounts of Wnt, loudly hinting that the protein's signals regulate early embryo development.

"We basically shut down every other project in the lab, and we've been pursuing Wnt ever since," says Moon, who heads the university's Institute for Stem Cell and Regenerative Medicine. "We were eager to know how Wnt signaling normally works—and what happens when it doesn't."

Today, Moon's long-term goal is to use Wnt signaling to coax stem cells into heart, brain, and other organs to replace, or regenerate, diseased cells. Rather than trying to grow whole limbs or sci-fi animals, Moon and a growing group of scientists focus on repairing existing tissue and organs.

"In the past, developmental biologists concentrated on the question of how you make an animal," says Douglas A. Melton, an HHMI investigator at Harvard University, who began in developmental biology before shifting his energies to stem cell medicine. "Now, researchers are returning to a question asked decades earlier: how do you *maintain* that animal?" He compares this shift in focus to working at a car repair shop, as opposed to a car factory. "We're beginning to appreciate the importance of maintenance, replenishment, and repair."

Response to Injury

AN ECLECTIC RANGE OF ORGANISMS—INCLUDING CRUSTACEANS, snakes, and salamanders—have evolved the ability to regrow lost tissues or limbs, whether from injury or natural biological cycles. Even humans share this talent to some degree: every day, the human body replaces an estimated 10 billion cells, including those in the liver, skin, and blood. But why do some species regenerate body parts handily, while others do not? Why can humans regrow a liver but not a pancreas? Can we borrow from nature's regeneration toolkit to treat human disease?

To address these questions, Moon and his colleagues have been documenting Wnt's mechanisms. Their studies show that zebrafish, tadpoles, mice, and potentially many other organisms respond to injury by turning on Wnt, a major signaling molecule. Wnt activates a cellular pathway, Wnt/ß-catenin, which launches the biochemistry of regeneration. Conversely, Moon's lab has found that another Wnt protein, Wnt5b, inhibits regeneration by launching a pathway that puts the brakes on regrowth signals.

In a study published online last December in *Development*, Moon's team demonstrated Wnt proteins in action: first they amputated zebrafish fins, and then they turned on or blocked regeneration of those fins by altering the activity of different Wnt proteins. In a related study, the researchers showed that *Xenopus* (African clawed frog) tadpoles also require Wnt activity to fully regenerate amputated limbs.

"We suspect that Wnt signaling is one of the earliest responses to injury in any form and is essentially a universal component of regeneration in animals," concludes Moon. "If we can fully determine the normal function of Wnt proteins, we might develop therapies for humans." By revving up certain Wnt proteins, for instance, researchers might one day replace brain cells lost to neurodegenerative disorders. Alternatively, by shutting off other Wnt proteins, they might treat certain cancers.

Boosting the Immune System

THE FIRST REGENERATION-INSPIRED THERAPY COULD BE TESTED IN humans as early as 2008. The lab of HHMI investigator Leonard I. Zon, a hematologist/oncologist at Harvard University, hopes to clinically test a therapeutic compound that eventually could allow doctors to regenerate a patient's immune system after damage by chemotherapy.

Zon's team recently screened a library of 2,500 chemicals to find small molecules that spark the production of blood

HHMI investigators Douglas Melton, Harvard University, Leonard Zon, Harvard University, and Randall Moon, University of Washington.

Melton: Joshua Dalsimer Zon: Kathleen Dooher Moon: Brian Smale

stem cells, a prerequisite for developing immune systems. The researchers discovered that prostaglandins—which, like hormones, regulate diverse chemical reactions—help to boost these stem cells. In particular, Zon's search identified a promising version of prostaglandin E2 (PGE2), made by drug manufacturer Upjohn in the 1980s.

To see how well the candidate PGE2 could help regenerate a damaged immune system, Zon's lab first irradiated zebrafish, essentially wiping out their native immune systems. When the researchers injected the irradiated fish with PGE2, the animals readily produced new blood stem cells. In related experiments on mice, they extracted blood marrow, bathed it in PGE2 for two hours, and found that the treated marrow at least doubled its rate of blood stem cell production for several months.

"This PGE2 derivative is the first known small-molecule mediator of stem cells and regeneration," says Zon. And that makes it a potential drug candidate. In a small but growing number of cases, doctors have successfully replenished a patient's weakened immune system by providing blood transplants from umbilical cord blood, which contains blood stem cells. In the future, physicians might instead administer a drug such as the PGE2 derivative. Joining forces, Zon and Moon are now studying how Wnt and PGE2, as well as their respective signaling pathways, work together to regenerate tissue in zebrafish.

Why Not the Heart or the Pancreas?

CANCER PATIENTS AREN'T THE ONLY ONES WHO COULD BENEFIT from regenerative therapy. This year, more than 1 million Americans will have a heart attack, according to the American Heart Association. An injured human heart cannot regenerate; instead, it scars. Too much scarring limits the heart's capacity to pump blood and can trigger abnormal heart rhythms, or arrhythmias.

While an HHMI investigator at Harvard Medical School, Mark T. Keating revealed, through a decade of studies in newts, mice, and zebrafish, that certain molecular signals enable specialized cells at the site of an injury to "dedifferentiate," or revert to stem cells, and then respecialize into the types of tissue needed to replace the lost or damaged cells.

Keating and colleagues at Children's Hospital Boston later uncovered some key biochemistry that naturally inhibits heart regeneration. In a 2005 study in *Genes and Development*, Keating's team revealed that an enzyme known as p38 MAP kinase suppresses rat heart cells, or cardiomyocytes, so they cannot multiply. When the team chemically inhibited this enzyme, however, the cells replicated in a petri dish. "Our work laid out some of the basic molecular mechanisms needed for heart regeneration," explains Keating, now a vice president and head of ophthalmology at the Novartis Institute of Biomedical Research in Boston. "I think we accelerated the field."

Indeed, Keating's work inspired ongoing heart regeneration research. His collaborators at Children's Hospital reported last year that rats injected with two drugs, to inhibit p38 MAP kinase and grow blood vessels, regained heart function after damage. That preliminary work continues.

Meanwhile, one of Keating's former postdoctoral researchers, Kenneth D. Poss, now a cell biologist at Duke University, is continuing the heart studies in zebrafish. While tiny—only a millimeter across and a microliter in volume—the zebrafish heart offers big lessons in regeneration basics. The Poss lab has developed a technique to clip off a piece of one ventricle—about a quarter of the chamber—and document how one population of cells, known as "progenitor" cells, effectively rebuilds the lost cardiac muscle in two months. Now, the lab is attempting to characterize the progenitor cells that launch heart regrowth.

While scientists work toward one day regenerating the human heart, another organ already regrows naturally: the liver. Even after surgeons remove almost two-thirds of the liver, remaining cells can rebuild a complete organ in three to six months. As they reported last January in *Nature*, Melton and his colleagues studied developing mice to compare, up close, the growth habits of the liver and its neighbor, the pancreas. Melton's long-term research aim is to learn how to generate pancreatic beta cells, which produce insulin, to cure type 1 diabetes.

His team applied two genetic techniques to alter the number of progenitor cells in the liver and pancreas of mouse embryos. In one set of experiments, they destroyed different numbers of pancreatic progenitor cells to challenge the pancreas. In a second set, they injected pancreatic progenitor cells into a strain of mice deficient in this cell type. Across all the experiments, the number of progenitor cells predicted the organ's final size: fewer cells, smaller pancreas; more cells, larger pancreas.

The liver, however, proved more adaptable. Its final size was normal, virtually regardless of the number of liver progenitor cells the researchers left in or injected into the embryos.

"Why the liver and not the pancreas?" asks Melton. The liver faces regular assault from the environment, in the form of alcohol and other blood toxins that the organ filters. Human skin also suffers damage, getting burned, scraped, and otherwise injured. Maybe, Melton speculates, these systems have evolved a way to endure frequent environmental insults by repairing themselves through regeneration.

Proceed with Caution

MEANWHILE, THERE MAY BE GOOD REASON WHY THE HUMAN BODY refuses to regenerate so many other tissues and organs, not to

> "We're beginning to appreciate the importance of maintenance, replenishment, and repair."
>
> **Doug Melton**

OUT ON A LIMB

In 1578, a British mathematician proposed an incredible boat called a submarine. Science fiction writer Jules Verne fantasized in 1865 that humans would fly to the moon. More recently, news reports have celebrated the idea that, with the application of some scientific knowledge, lost human limbs might grow back. Will this sensational idea, like others before it, come true?

Even regeneration enthusiasts hesitate. “It’s reasonable to think about regenerating specific cell types, like neurons,” says HHMI investigator Alejandro Sánchez Alvarado, a molecular biologist at the University of Utah. “But something as complex as a hand? Before we even embark on that in humans, we need to do so much more.”

Sánchez Alvarado has spent the past decade studying an invertebrate model of animal regeneration: a freshwater flatworm called *Schmidtea mediterranea*, or planaria. This flatworm can fully regenerate from a fragment as tiny as 1/279th of the original organism.

Sánchez Alvarado and his colleagues have begun to unveil the flatworm’s regenerative machinery in detail, using RNA interference—a technique that systematically silences targeted genes to determine their function. In 2005, his lab conducted the first large-scale gene inhibition study of planaria, as reported in *Developmental Cell*. Of 1,065 genes screened, the team identified 240 associated with specific developmental processes or defects. In particular, they identified cells that potentially regulate stem cells, regeneration, and homeostasis—a cell’s dynamic equilibrium.

Building on that work, the lab is now deconstructing seven major signaling pathways in planaria, all known to govern cell signals. “How do progenitor cells work?” asks Sánchez Alvarado. “When are they on and off? Which cells activate them—and in turn, what types of cells do progenitors activate?” The advantage of the fast-growing flatworm, he says, is that experiments to address these questions can be performed more quickly than in vertebrate models such as the zebrafish.

“Regeneration is more than just early development played out again in maturity,” Sánchez Alvarado adds. “The same basic protein players are at work, but their regulation is different. And that’s what makes it fascinating.” *– K.B.*

mention limbs. Unchecked regeneration resembles the runaway cell growth that characterizes cancer. In a study published May 18 in *Science*, Moon and collaborators reported evidence for concern.

Some proteins act as cellular brakes, regularly shutting down Wnt signaling and other cell-regeneration pathways. This “stop” mechanism keeps cell populations in check. For instance, scientists have documented that, in colorectal cancer and melanoma, mutations disrupt proteins that normally turn off Wnt.

Moon’s team explained how this biochemistry might play out in Wilms’ tumor, a form of pediatric kidney cancer. Through their investigations, the researchers discovered the potential involvement of WTX, a protein that normally acts as a tumor suppressor by degrading a protein network that activates the Wnt pathway. Working in zebrafish, frogs, and cultured cells, the team found that Wilms’ tumor mutates the gene that encodes WTX, thus disabling the protein. Without WTX as a brake, the Wnt pathway is activated more than usual, triggering harmful cell growth that becomes a tumor.

Thus, although regenerative medicine has great potential, researchers looking for dramatic outcomes in humans might well proceed with caution, lest they re-create, in one way or another, Moon’s surprise results long ago in tadpoles.

There may be a safer and more practical application of regeneration research—the familiar human condition of degeneration, which, according to Melton, is the flip side of regeneration. On that note, Moon and colleagues published a May study in the *Proceedings of the National Academy of Sciences* demonstrating that a single amino acid change in a protein that works with Wnt is associated with late-onset Alzheimer’s disease. Moon concludes that Wnt biochemistry is all about balance. Too much—or too little—Wnt affects both regeneration and degeneration.

“We increasingly suffer from diseases of degeneration,” Melton notes. “Diabetes, neurodegenerative disorders, cardiovascular disease. All have features in common: an unknown environmental stimulus, many genes, and a long time between cause and effect. If we’re interested in slowing degeneration, we should be focusing on how the body maintains and repairs itself.” ■