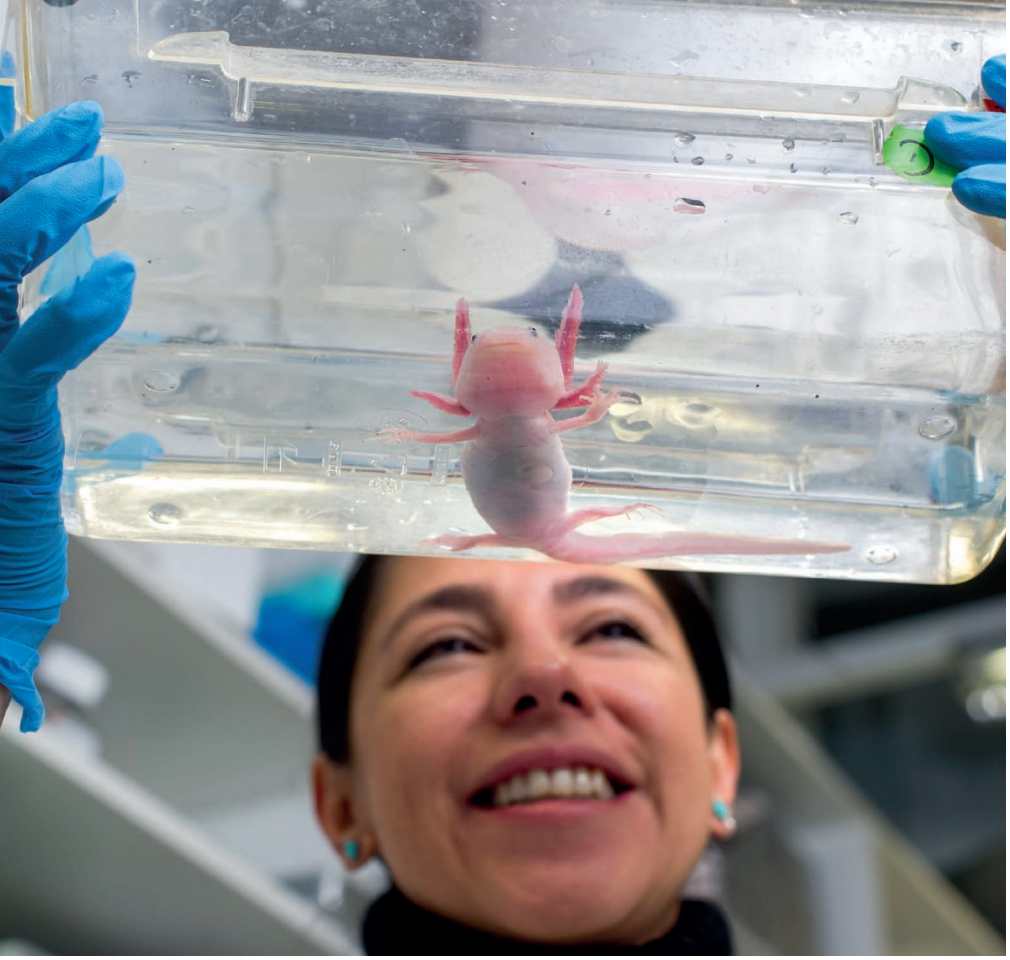

ROBERT MICHAEL/AFP/GETTY

The Mexican salamander (or axolotl) can regrow limbs, making it an ideal model for studying regeneration.

ANIMAL MODELS

Unlock your inner salamander

Some animals can regrow body parts with ease. Biologists hope to figure out their secrets and apply them to humans.

BY SUJATA GUPTA

Picture this: a salamander and a mouse both lose a limb in an accident.

"A salamander laughs at that kind of injury," says James Godwin, an immunologist and regeneration biologist at The Jackson Laboratory in Bar Harbor, Maine. "For a mouse, it's devastating."

That's because, over the next few weeks, the salamander will regrow that lost limb — a perfect replica of the original — without any scar tissue forming. Meanwhile, the mouse, if it survives, will be left with a stump.

Researchers have long looked at these divergent trajectories and wondered whether it might be possible to make mice — and by extension humans — behave more like the salamander. This requires identifying the molecular and cellular processes activated during the regenerative process that allow animals as varied as zebrafish, lizards and certain species of rodent to regrow everything from limbs to organs. The hope is to one day insert or reactivate the same pathways in humans.

It's a tantalizing prospect. "Salamanders rebuild the tissue so perfectly that you can't recognize that it was regenerated," says Elly Tanaka, a regenerative biologist at the Research Institute of Molecular Pathology in Vienna.

Wouldn't it be amazing, she says, if humans could do the same?

LOST ABILITY

The origin of animals' ability to regenerate limbs and organs is uncertain. One possibility is that as mammals evolved more complex immune systems, they developed a scarring response to injury that helped to protect them against infection.

Essentially, says Kenro Kusumi, a regeneration biologist at Arizona State University in Tempe, an injured animal must 'choose' between a quick heal with scar tissue or a slow regenerative response that might leave it more vulnerable to predation for a few weeks. Although there are exceptions, mammals generally go for the quick fix, whereas salamanders and a few other vertebrates, such as fish and lizards, hunker down and rebuild.

If mammals have gained the scar response at the expense of regeneration, they might still have the molecular and cellular pathways needed for regeneration lying dormant. Regeneration in humans, says Kusumi, might "just be a matter of turning the switches back on."

Humans, particularly early in life, do have limited repair capabilities, suggesting that some of the genetic programs for regeneration are present in mammals, but get turned off during development.

Consider the heart. When an adult has a heart attack, hundreds of millions of heart muscle cells die and a scar forms around the injury. With no way to regenerate or repair those cells, the weakened and scarred heart is more prone to subsequent heart attacks or failure. But research shows that newborn mice can regenerate heart-muscle cells. If you take a pair of scissors and snip off a third of the ventricle of the heart in a one-day-old mouse, says Godwin, the heart will repair itself without scarring. Do the same experiment at day seven, however, and that ability has been lost.

Intriguingly, human babies might have the same capacity. One case study reported an infant who had a heart attack at birth, and whose heart was scar-free a year later[1]. Nevertheless, evidence is sparse and experiments are obviously unethical.

PUTTING PARTS TOGETHER

To achieve regeneration in humans, scientists must first identify the switches that turn on regeneration in other animals. One candidate for studying this is the salamander, which is capable of regrowing limbs that are perfect replicas of the original. That's why Tanaka has been studying the amphibians for 20 years. "The salamander system provides a kind of guiding principle of how regeneration can work," she says, "of how parts are put together".

But it is not the only candidate. Over the years, regeneration biologists have expanded their animal repertoire, and their choice is often driven by the regenerating body part of interest. For instance, Kenneth Poss, a regeneration biologist at Duke University in Durham, North Carolina, works with zebrafish (*Danio rerio*) because he's interested in heart regeneration. Although other animals, such as salamanders and lizards, are also capable of rebuilding a heart, the main advantage of the zebrafish is that

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it is an established lab animal, which makes it easier to create mutant and transgenic strains to test theories, Poss says.

Some researchers are turning their attention to lizards, which can generate new tails. Although lizards are much closer to humans, genetically speaking, than salamanders or zebrafish, the new tail is imperfect, composed entirely of cartilage and no bone. Even so, figuring out how to grow back cartilage in humans would be a huge feat. "Cartilage is not a tissue that most animals can even heal," says Thomas Lozito, a developmental biologist at the University of Pittsburgh in Pennsylvania, whose work compares regeneration in lizards and salamanders.

"Less than 0.2% of mammal species have ever been surveyed for regenerative abilities."

Thanks to efforts such as these, the basic steps involved in regeneration have been established in the organs of several types of animal; these include the zebrafish heart, salamander limb and mammalian liver. Although still a controversial idea, many scientists now think that regeneration is driven by a process known as dedifferentiation. That is, after an amputation or injury, adult fibroblast and muscle cells elsewhere in the body seem to receive signals to revert to a more embryonic state. "The cells go one step back in development," says Guo Huang, a regenerative biologist at the University of California, San Francisco.

As that dedifferentiation takes place, epidermal skin cells migrate to the injury site to seal it by forming a wound epidermis. Cells at the wound site then form a ridge, or apical epithelial cap, which sends out signals for the dedifferentiated cells to migrate in. Soon, the dedifferentiated cells start dividing to populate the bud of a new limb or other body part, known as a blastema.

Scientists have begun to identify and even manipulate the genes and proteins activated during regeneration. "We've identified enough molecules that we can kind of make a basic mechanistic description of what's happening in the salamander during regeneration," says Tanaka.

The next step, researchers say, is to put these genes back in lab rodents. Getting humans to regrow an amputated arm is still a long way off, but researchers suspect that this intermediate step could happen within the next few years. "Once you translate a finding from a salamander or a fish to a mouse," Godwin says, "you've got a better chance of translating it to humans."

THE MISSING 99.8%

Perhaps that intermediate step in salamanders, lizards or fish will prove extraneous as researchers find mammals that are capable of the same sort of regeneration. Working in mammals would also circumvent the logistical problems that come with translating findings from distantly related species to humans.

Ken Muneoka, a regeneration biologist at Texas A&M University in College Station has been making this argument since the 1980s. Humans are very different from other creatures, Muneoka says. Whereas a salamander limb is mostly cartilage, a human limb contains a lot of bone. So any regenerative response in the mammal must first account for how to deal with big chunks of exposed bone. "That is very specific to the mammal," Muneoka says.

Some mammals do seem to have sorted out at least part of the secret to regeneration, however. Consider the story of the African spiny mouse.

In 2009, Ashley Seifert, a newly minted developmental biologist, spent the summer in Kenya. An acquaintance who was familiar with the country had told Seifert about a local rodent that could lose — and regrow — huge chunks of skin. Intrigued, Seifert set up some traps and captured several spiny mice (*Acomys*), which are abundant in the rocky outcroppings that pockmark the savannah. He quickly discovered that even just handling these rodents caused their skin to tear. Initially, the skin-healing process looked similar to that of other rodents, but after about 20 days, rather than the typical hairless scar, the spiny mice started to regrow hair follicles. In fact, the skin never scarred at all.

"When we conducted the initial experiments in the field, we watched the animals heal over time and regenerate the tissues," says Seifert, now at the University of Kentucky in Lexington. In a process known as autotomy, these rodents seem to be casting off body parts to evade a predator — the same sort of self-preservation method seen in lizards and salamanders. Seifert had found a mammal that seemed to have turned its regenerative switch for skin back on[2]. Seifert discovered that the mice could also repair holes in their ear — a classic body part in which to study wound healing — including regenerating skin, hair, muscle and even cartilage. His research (along with Muneoka's) suggests that the mice regenerate by first developing a structure that bears a strong resemblance to the blastema seen in salamanders and other regenerating vertebrates. This supports the idea that regeneration in mammals such as the spiny mice evolved in the same manner as regeneration in salamanders.

Seifert's finding raised the enticing possibility that there may be other mammals that have abilities like the African spiny mouse. "There's around 5,300 mammal species on Earth right now," Seifert says. "Less than 0.2% have ever been surveyed for regenerative abilities."

GETTING TO HUMANS

Regenerating lost cartilage or appendages in humans is still decades or more away, but various lines of research suggest that turning those ancient regenerative switches back on in humans may come sooner.

Perhaps the most exciting clues for doing this comes from Muneoka's work in rats — specifically their digit tips. It's been known for at least half a century that when a child severs a finger above the top-most joint, that the fingertip (bone, flesh, nail and all) will typically grow back. This ability seems to become weaker with age, although teenagers and adults have also been known to grow back digit tips. Nobody knows for sure why this happens, but some suspect that the nail creates an environment that is conducive to regeneration or that mammals evolved the ability to regenerate in this region because the digits are crucial to limb use and survival. Whatever the reason, Muneoka wants to know how it happens.

Using mice, who display a similar phenomenon, Muneoka has shown that the gene *Msx1* controls a signalling protein known as Bmp. Remove either *Msx1* or Bmp and mice lose the ability to repair their digit tips. He has also found that adding Bmp stimulates partial regeneration of digits severed below the first joint[3].

"We have shown that you can enhance the regenerative properties of mammals," Muneoka says.

For Muneoka, that is proof of concept that regenerative capabilities can also be enhanced in humans. Initially, he suspects, the process will work better in children than in adults and will be limited to digits rather than entire limbs. "I believe that with adequate funding, human-finger regeneration in children will be possible within 20 years," he says. We may never catch up with the salamanders, but at least we're off the starting blocks. ■

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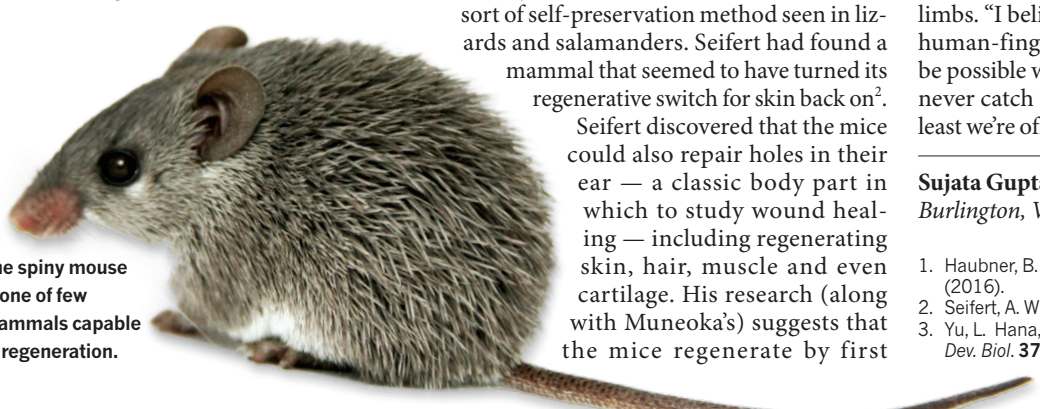
1. Haubner, B. J. *et al*. *Circulation Res.* **118,** 216–221 (2016).
2. Seifert, A. W. *et al*. *Nature* **489,** 561–565 (2012).
3. Yu, L. Hana, M., Yana, M., Leea, J. & Muneoka, K. *Dev. Biol*. **372,** 263–273 (2012).



ASHLEY SEIFERT

The spiny mouse is one of few mammals capable of regeneration.