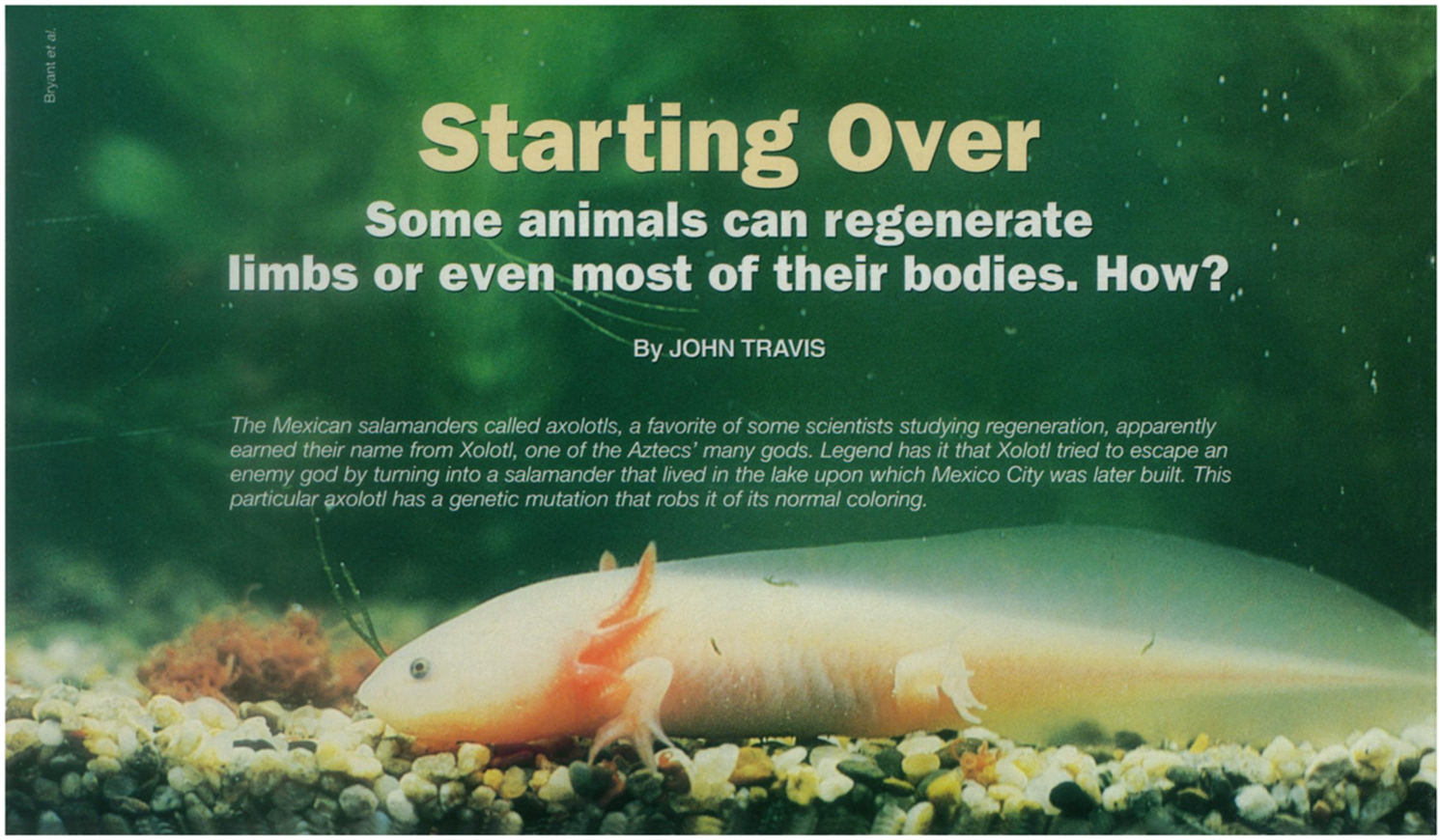


Starting Over

Some animals can regenerate limbs or even most of their bodies. How?

By JOHN TRAVIS

The Mexican salamanders called axolotls, a favorite of some scientists studying regeneration, apparently earned their name from Xolotl, one of the Aztecs' many gods. Legend has it that Xolotl tried to escape an enemy god by turning into a salamander that lived in the lake upon which Mexico City was later built. This particular axolotl has a genetic mutation that robs it of its normal coloring.

Last summer, on the city's hottest day in a century, a construction platform beneath a Washington, D.C., bridge collapsed, dropping a 15-ton steel beam that killed one man and pinned a second by his legs. With a blowtorch, rescue personnel eventually freed the injured man and rushed him to a hospital, where physicians had to amputate his left leg and transplant a back muscle into his right leg.

Such misfortunes have long prompted physicians to dream of restoring damaged or amputated limbs by inducing them to regenerate. Other injuries have spurred doctors to imagine regrowing crushed spinal cords and dead heart tissue.

These medical flights of fancy draw inspiration from the well-known ability of certain animals to perform precisely those feats. Consider the urodeles, a class of vertebrates that includes newts and salamanders. These animals possess an enviable talent for regrowing arms, legs, tails, heart muscle, jaws, spinal cords, and more. Some simpler organisms can even be sliced and diced, with each piece giving rise to a complete new animal.

Surprisingly, given the obvious medical appeal of regeneration, scientists know relatively little about the process. Researchers have produced detailed descriptions of regeneration, but they don't understand the molecular signals driving this physiological tour de force.

The problem lies largely in the animals that scientists have traditionally chosen to study, such as mice, frogs, fruit flies, and nematodes. Though investigators have amassed a wealth of knowledge about these common laboratory animals and have many ways to study and manipulate them genetically, the adults of these species don't come close to matching a urodele's regenerative powers.

While most scientists have tried to overcome the mysterious barriers to regeneration that exist in the traditional laboratory animals, a few investigators have taken the road less traveled. They have decided that to unearth the genes and proteins vital to regeneration, they must study animals that can actually regenerate.

"We're trying to understand the molecular basis of regeneration," says Alejandro Sánchez of the Carnegie Institution of Washington in Baltimore.

The basics of limb regeneration have been evident for more than a century. First, the animal heals the wound at the site of the missing limb. Then, various specialized cells at the site, such as bone, skin, and blood cells, lose their identity in a process called dedifferentiation. The resulting blastema, a mass of unspecialized cells, proliferates rapidly to form a limb bud. The cells ultimately take on specialized roles as the new limb takes shape.

"Salamanders can turn back time," says David L. Stocum of Indiana University-Purdue University in Indianapolis. "The trick they have is dedifferentiation of mature cells. They regenerate a lot of tissues this way."

For some reason, people and other nonurodele vertebrates lack this ability to create a blastema. They repair a wound and stop. Rather than rely on dedifferentiation, the few human tissues that can regenerate—such as blood and the liver—turn to a small number of unspecialized cells set aside during embryogenesis. These so-called stem cells maintain the ability to proliferate rapidly and indefinitely.

In recent years, Susan V. Bryant and David M. Gardiner, both at the University of California, Irvine, have turned to salamanders called axolotls to study how a blastema transforms itself into a limb. The process depends on many of the same genes employed when an embryo originally creates a limb, but the blastema doesn't turn those genes on and off in exactly the same pattern.

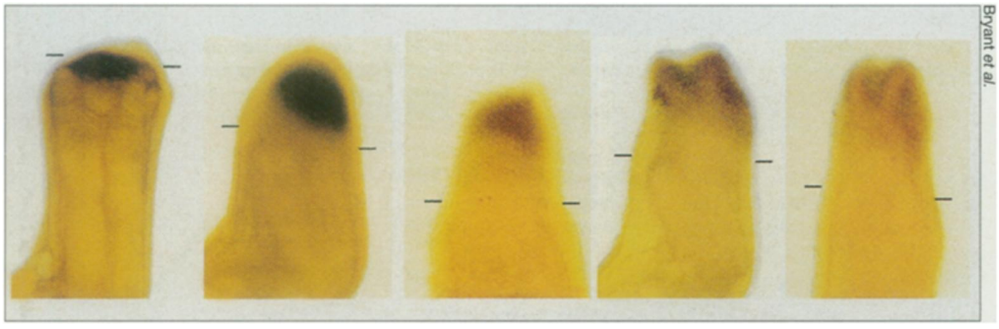
Take the *HoxA* genes. From developmental studies of many animals, scientists have shown that this cluster of genes helps pattern a growing limb. Which *HoxA* genes in a cell turn on ultimately determines the cell's position in the final appendage.

Remarkably, the order of the *HoxA* genes on their chromosome mirrors the genes' order of activation. In the developing limb bud of the arm, for example, *HoxA* genes at one end of the chromosome turn on first, marking cells intended for the upper arm. As the bud grows, successive *HoxA* genes become active, identifying cells destined to form the lower arm. Finally, the *HoxA* genes farthest down the chromosome take their turn, signaling which cells will become part of the hand.

Bryant and Gardiner have found that this *Hox* code exists in regenerating

axolotl limbs, but with a twist: The timing of gene activation does not follow the genes' order on the chromosome. For example, after a limb is amputated, both the *HoxA-9* and *HoxA-13* genes become active a day or two into the 3-week regenerative process. In the embryo, *HoxA-13* follows *HoxA-9* and isn't turned on until the very end of limb patterning.

The blastema first specifies which cells will form the axolotl "hand," concludes Bryant. "Then, it's a process of filling in the gap. That allows you to make exactly what's missing." Studies of another developmental gene cluster, the *HoxD* genes, bolster this view, she adds.



Bryant et al.

These images show early to middle stages of forelimb regeneration in axolotls. The extent of regrowth can be measured from the hash marks, which indicate where the original limb was amputated. A close look at the limb tips in the later images shows that digits of the new limb have started to form. The dark staining marks areas where the HoxA-13 gene is active.

Axolotls have shed light on another curious difference between developing and regenerating limbs. In the embryo, limbs take shape well before nerves arise. Yet a denervated axolotl limb can't initiate the process of regeneration, says Bryant. Moreover, scientists can denervate a regrowing limb midway through regeneration without stopping it, suggesting that there's a window of time in which regeneration is dependent on the nerves.

Investigators have speculated that nerves release some vital regeneration factor, but its identity has proved elusive. Recently, Bryant, Gardiner, and their colleagues found the axolotl version of a fruit fly gene called *distalless*, or *dll*. In flies, the gene is needed for proper leg development.

This gene turns on in a regenerating axolotl limb just as the limb loses its dependence on nerves for regrowth, the investigators reported in the November 1996 DEVELOPMENT.

They also discovered that denervating a regenerating limb before this transition prevents *dll* from turning on, whereas later denervation does not turn off the gene. Suspecting that limb regeneration requires *dll* activity, the investigators sought to determine why the gene fails to turn on after early denervation.

They eventually hit upon a protein called FGF-2. When they implanted beads coated with FGF-2 into a regenerating limb, *dll* activity stayed normal after early denervation. The scientists further established that nerves indeed make FGF-2.

While a limb blastema can produce its own FGF-2, the scientists believe it needs an initial supply from nerves before it becomes self-sufficient. "We think the nerves provide the FGF-2 to prime that transition," says Bryant.

Consequently, an absence of nerve-derived FGF-2 may be one of the barriers to limb regeneration in most vertebrates. Triggering an amputated arm to regrow will not be simply a matter of providing this growth factor, but FGF-2 may provide part of the eventual recipe for regeneration in people.

"The hope is that there won't be too many things to replace and that you can get to the point where the [regeneration] cascade begins to roll," says Bryant.

Sánchez loves axolotls. In fact, he keeps two as pets in an office aquarium. Yet Sánchez believes the salamanders will keep secret most of their regenerative tricks.

The inability to mutate specific genes in axolotls and other urodeles, or to add specific genes to the animals, hinders attempts to determine the significance of any new gene that researchers suspect of aiding regeneration.

Though the work of Bryant and her colleagues is "heroic," says Sánchez, "you reach a ceiling of how much you can do with the organisms."

"The genetics [of urodeles] is poor, their generation time is long, and there are no transgenics yet. That's why it's a struggle for people to keep working in the area," acknowledges Bryant.

Consequently, Sánchez intends to pursue his regeneration studies primarily with planaria, simple worms that have their own legendary regenerative ability. Chop one of the inch-long creatures into 300 pieces, and in a matter of days 300 planaria will be swimming around. Indeed, Sánchez' group occasionally uses that strategy to expand the worm colonies.

Sánchez' plan is to identify the genes that enable planaria to regenerate. Why? "Most likely we have those genes as well," he says.

That statement would have sounded brash 20 years ago. Yet scientists studying worms, primarily those called *Caenorhabditis elegans,* have increasingly found that genes from these simple animals resemble genes in more complex creatures. In addition, the related genes are frequently used in a similar manner.

As a result, Sánchez is operating on the assumption that the regeneration strategy used by planaria resembles the one employed by urodeles and may provide insight into the human failure to regenerate. There's some evidence supporting this contention. When cut in half, the worms form blastemas similar in architecture to those seen when researchers amputate the limbs of a urodele.

From Hydra to hydras

Tales of regeneration, true and false, go way back. Hercules struggled to slay the Hydra, the many-headed monster that grew two heads for every one lopped off. Aristotle documented the ability of lizards to regrow tails, notes science historian Charles E. Dinsmore of Rush Medical College in Chicago. Aristotle propagated myth almost as often as the truth, however. The Greek philosopher also wrote of baby birds regenerating eyes, adds Dinsmore.

Regeneration began to take a more serious scientific bent in the mid-18th century, thanks largely to Abraham Trembley's investigations into the hydra, a plantlike aquatic animal that can form two whole organisms if split in half. In 1767, Lazzaro Spallanzani reported the ability of salamanders to regenerate limbs, not just their tails.

This early regeneration research, notes Dinsmore, ignited fierce controversy centering on whether the regenerated limb or tail had existed in a miniaturized form inside the animal or whether it somehow arose from unspecialized living matter. Nonscientific issues, such as whether the soul could split in two, also emerged from the lively discussions on the research. Regeneration "became a political, social, and theological issue, which kept the light on it," says Dinsmore. —*J.T.*

Planarian regeneration does have clear differences, however. The most significant seems to be that the worms do not dedifferentiate cells to create the blastema. Instead, planaria turn to cells called neoblasts.

Scattered within the planarian body, neoblasts apparently remain in an unspecialized, stem cell state, which enables them to differentiate into any cell type. Wherever planaria are cut, the neoblasts migrate to the site and form a blastema by themselves.

Sánchez' colleague Phillip Newmark has kept planarian neoblasts alive in petri dishes for several weeks, an advance the researchers hope will allow them to genetically alter the animals. The investigators will try to slip genes into the neoblasts or mutate existing genes, then add the cells to worms whose own neoblasts have been destroyed with radiation. When such worms regenerate, any new cells should derive from the genetically engineered neoblasts, says Sánchez.

Efforts to identify planarian regeneration genes have already offered some promising leads, Sánchez' group reported at last summer's International Congress for Developmental Biology in Snowbird, Utah. With a technique called subtractive hybridization, the researchers collected fragments of genes turned on in blastemas when a planarian regenerates its tail, its head, or both and compared them to genes normally active in the head and tail. Genes active only in the blastemas presumably participate in regeneration, notes Sánchez.

The investigators detected dozens of DNA fragments specific to regenerating blastemas, some belonging to genes that become active in the initial hours of the process. In several cases, the researchers have found that the fragments are parts of genes found previously in other animals.

One such gene encodes an enzyme that degrades the extracellular matrix, a mesh of proteins and other molecules that surrounds cells. This enzyme may trigger regeneration by releasing growth factors bound to the matrix or by eliminating obstacles to a cell's proliferation and movement, says Sánchez.

Is the notion of regenerating human limbs or diseased tissue a pipe dream or a realistic expectation for the 21st century? It's far too early to know whether there are insurmountable differences between animals that can regenerate and those that cannot, says Jeremy P. Brockes of University College London.

He and his colleagues have recently shown that some factor present in blood serum, newt or any other kind, can induce newt muscle cells to dedifferentiate. The muscle cells of nonregenerating vertebrates don't respond to the serum, however, indicating that they are not sensitive to this still unidentified factor.

If feasible, overcoming this insensitivity might prove invaluable. Cardiac specialists might then learn to trigger damaged heart muscle to replace itself. Such an advance could be possible even if scientists never obtain sufficient expertise to regenerate complex structures like limbs, suggests Brockes.

Given the potential value of regeneration in medicine, Stocum finds it puzzling that so many scientists choose to study cell and organ transplantation or work on building artificial hearts, livers, and limbs rather than investigate animals that can regrow parts of themselves.

"With regeneration, you don't have to worry about [finding] donor cells, and you don't have to worry about immune rejection," he says. "Regeneration is clearly superior to any kind of transplantation or artificial tissues." □

To find a link to an animated version of limb regeneration, visit SCIENCE NEWS ONLINE *at* ***http://www.sciencenews.org***.

Nutrition

Getting older—and a little rounder?

A person's body fat typically doubles between the ages of 20 and 50. While this middle-aged spread is usually attributed to eating too much and exercising too little, there may be more to it than that. The body's ability to break down and use large quantities of fat drops with age, a new study finds. As a result, more of the fat eaten at large meals will become body fat instead of being burned as energy, explains Susan B. Roberts of the Department of Agriculture's Human Nutrition Research Center on Aging at Tufts University in Boston.

On four different days, her team analyzed how 16 women—half of them in their twenties, the rest over 60—metabolized various portions of peanut-butter-and-jelly sandwiches and milk. The four test meals ranged from a 250-calorie snack to a 1,000-calorie feast that represents two-thirds of the average 60-year-old woman's daily energy intake.

The two groups processed the fat in the smaller meals with equal efficiency. However, the older diners burned only 70 percent as much fat from the big meal as those in their twenties, the researchers report in the October AMERICAN JOURNAL OF CLINICAL NUTRITION. One explanation may be the fact that older women possess more glucagon, a hormone that instructs the body to put more sugar into the blood. "That's not good," Roberts says, because the more readily used sugar discourages the body from burning fat. In their thirties, people begin to lose skeletal muscle (SN: 8/10/96, p. 90), the site of most fat metabolism.

To cope with these age-related changes, Roberts suggests, older diners should not only eat less at a sitting but also exercise more—to strengthen and retain fat-burning muscle. —*J.R.*

Boning up on fish oil

For decades, nutritionists have extolled the virtues of fish oils and other foods rich in omega-3 fats as a means of keeping the heart healthy. Studies in animals now suggest that this type of fat also aids bone. If the same holds for humans, physicians may one day add omega-3 fats to the dietary regimen they prescribe to help stave off osteoporosis.

Most polyunsaturated fats fall into one of two groups: the omega-6 fats found in corn oil and the omega-3s found in ocean fish, flaxseed, green vegetables, and some nuts.

In recent years, the omega-6 content of Western fare has been climbing—often at the expense of omega-3, which now constitutes around 7 percent of polyunsaturated fats in the typical diet, notes Bruce A. Watkins of Purdue University in West Lafayette, Ind. At an international omega-3 conference in Bethesda, Md., last month, he reported on his studies of chicks and rats. As omega-3 dropped from 50 percent of polyunsaturates to some 12 percent, bone formation also fell—by about 20 percent.

The fat's apparent stimulation of bone growth seems to arise from its effect on at least two hormonelike compounds. When omega-3 makes up only 12 percent of polyunsaturates in the diet, production of prostaglandin E (PGE_2), a "local hormone," is high, limiting bone growth, Watkins says. Increasing the omega-3 share of polyunsaturates to 50 percent halved the bone-making cells' production of PGE_2, bringing it to a level more conducive to bone formation.

The same dramatic increase in omega-3 fats enhanced the activity of insulinlike growth factor (IGF) by 50 percent and increased significantly the production of a protein that ferries IGF around the body, Watkins' data show. Production of IGF, which fosters continual growth and remodeling of bone, typically falls in old age, precipitating the bone loss underlying osteoporosis.

"I think the message here," Watkins told SCIENCE NEWS, "is that when the diet supports more responsive remodeling, you can shape the bone to be stronger." —*J.R.*