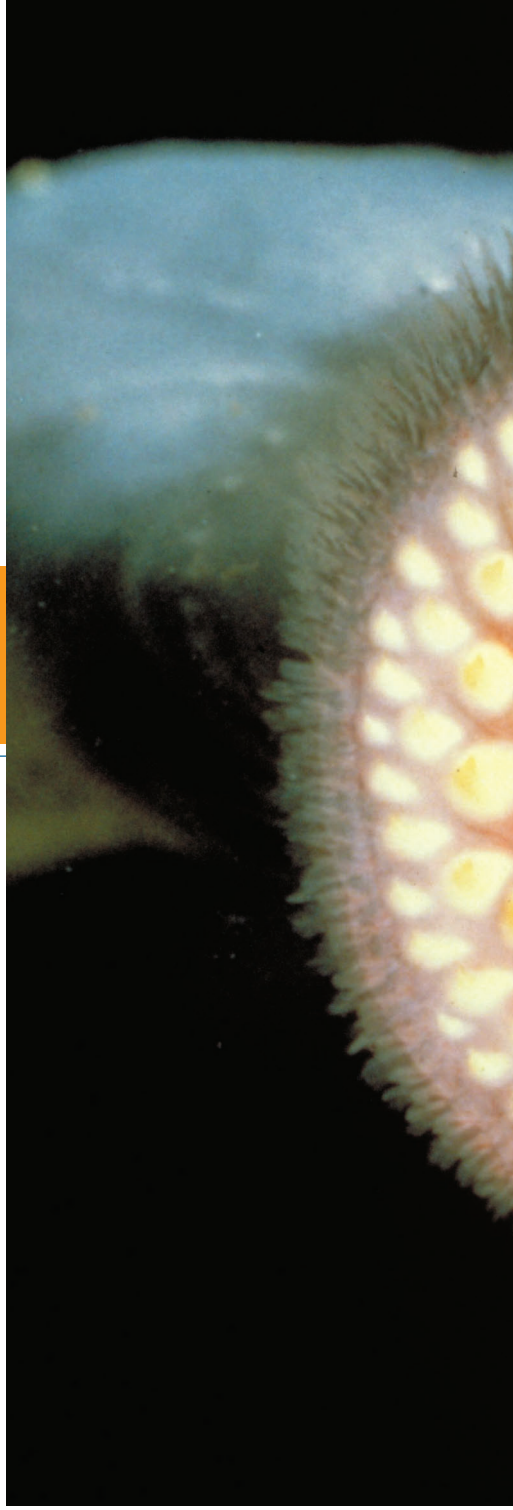
Jaws 'R' Us

By Kathy Svitil



You'd be hard pressed to find an organism that's as unpleasant as *Petromyzon marinus*, the sea lamprey. Its looks alone are sure to turn heads . . . *away* . . . with its suction-cup-like, jawless mouth and concentric rings of sharp, curved teeth. And this three-foot-long, eellike creature's behavior is even worse. A parasite, the lamprey latches onto its prey, rasps away scales, skin, and other tissue, and slurps out the meat and blood. Nasty.

And yet, this uncongenial life-sucking horror is turning heads among biologists. It may offer insight into some of life's biggest mysteries—the origin of backbones, the development of jaws, and even how we higher vertebrates might have evolved the large brains that let us dominate our world. (A lot to lay at the feet of an animal that doesn't actually *have* feet.)

Every summer, researchers from around the world flock to the laboratory of Caltech biologist Marianne Bronner to study lamprey embryos. Like salmon, lampreys are born in inland rivers and eventually migrate to the sea (and in the United States, to the Great Lakes, where they're a scourge to native fish populations—and the rare unlucky swimmer). When they reach sexual maturity, around age seven, lampreys return to freshwater rivers and streams to breed. They essentially turn themselves, Bronner says, into "giant bags of gametes," or eggs and sperm, which are released into the water. Then the lampreys—deflated—digest themselves from the inside out, and die.

Although the parasitic animals are, by definition, pests, "their embryos are really interesting," Bronner says.

Her esteem for the lamprey comes in large part from the window this creature opens into her true research passion: the neural crest. In the embryos of lampreys and other vertebrates—animals with backbones—neural crest cells appear very early on in the budding nervous system's development, then migrate away and transform into a host of other structures, including the skeleton of the face, many types of nerve cells, adrenal glands, and pigment cells in the skin. In contrast, these cells don't exist in animals without backbones.

"Why do vertebrates have these neural crest cells? How was this cell type invented? And why would cells that come out of the central nervous system give rise to all these different non-nerve-cell types?" Bronner asks. To help answer those questions, she and her colleagues have tracked developing neural crest cells in the embryos of a variety of different vertebrates—including lampreys—and pinpointed a number of critical genes.

One gene, dubbed *AP2*, is crucial to the formation of your facial bones and nerves, both of which are derived from the neural crest. However, Bronner and her colleague Daniel Meulemans (PhD '04) have found that *AP2* also shows up in the neural crest-less amphioxus, an invertebrate relative. Amphioxus, or lancelets, are also eellike, but they're only about two inches long and can be found buried in the sand in shallow tropical seas. Though they are boneless, their embryos have a nerve cord that resembles our developing spinal cord, making them our nonvertebrate cousins on the chordate family

Meet *Petromyzon marinus*, the sea lamprey, in all its faceless glory. Instead of a mouth, the head end of a lamprey sports a wide, flat sucker equipped with rows of tiny teeth.

tree. In amphioxus, as in vertebrates, *AP2* is turned on very early in development, in the epidermal cells that eventually become skin. But then the gene turns off for a time, and when it comes back on, it behaves very differently. Instead of being active in neural crest cells, as it is in vertebrates, it turns up in an organ called the cerebral vesicle, which is amphioxus's primitive excuse for a brain. That suggests, Bronner says, that although the early job of *AP2* is similar in all chordates, "the gene gets turned on in a new place in the vertebrates, later in development."

The neural crest genes play different roles even among vertebrates themselves. For example, Bronner and her colleague Tatjana Sauka-Spengler have found that although almost all of the genes usually activated in the neural crest cells are turned on in lampreys, they are not always expressed at the same *time*.

"That's one of the reasons we want to look at the jaw," Bronner says. The lamprey's head contains neural-crest-derived cartilage that becomes facial structures in other vertebrates, but the faceless lamprey has no jaw. "We're trying to find out why, during vertebrate evolution, another derivative of neural crest cells was invented that gave rise to jaws."

Lampreys have been on the scene for 550 million years, so being jawless clearly hasn't hurt them much. But when jaws did appear, vertebrates really took off. It's hard to know what sparked the explosion of new species, Bronner says, "but you have to eat a lot less if you eat meat instead of plankton. Jaws are important because they allowed predation to occur, and there's some speculation that *that* allowed the brain to grow, and made vertebrates evolve even faster.

"These lampreys have really nice migrating neural crest cells that come out of the nervous system, and they go to the same places that neural crest cells would in us," Bronner says, "except they are not remodeled into the bones of the jaw." Furthermore, lampreys aren't *just* missing a jaw—they lack other common crest-cell derivatives such as sympathetic ganglia, which are clusters of neurons that prepare your body to fight or flee by doing such things as controlling the blood supply to your muscles.

Determining where these features come from requires decoding the genetic playbook that directs the differentiation of cells derived from the neural crest. "We want to see what is different in the lampreys' instructions compared to other organisms," Bronner says. To do that, she and her colleagues have recently turned to yet another organism: the tiny, tropical, transparent zebrafish. Zebrafish are used by many developmental biologists because they grow rapidly in the lab—and

A THREE-WAY SPLIT

Nüsslein-Volhard and Wieschaus shared their 1995 Nobel with Caltech's Ed Lewis. The Morgan Professor of Biology, Emeritus, at the time of his death in 2004, Lewis had been at Caltech as a student (PhD '42) and faculty member since 1939. Lewis, Nüsslein-Volhard, and Wieschaus all induced genetic mutations into the common fruit fly, *Drosophila melanogaster*, to determine the affected genes' roles in the insects' development.

Nüsslein-Volhard and Wieschaus discovered a handful of master genes that control which end of the fly egg becomes the embryo's head and that lay out the basic body plan. Meanwhile, Lewis found a second group of master genes that orchestrate the development of the body's parts: eyes, antennae, wings, legs, what have you. In work that united genetics, developmental embryology, and evolution, Lewis showed that these genes are strung along the chromosome in the same order, from head to tail, as the parts they control—a basic organizational scheme since found in all other animals. These homeotic or HOX genes, as they're called, are very, very old, containing almost identical DNA sequences in flies and in humans.

because you can peer into their see-through bodies.

"How that work began is one of those 'only at Caltech' stories," explains Bronner. At the time, about six years ago, the National Institutes of Health was creating so-called Centers for Excellence in Genomic Science. "These grants were tailored for projects that were considered very risky but potentially had very high yield," she recalls. "I thought, Who would be fun to work with, and what kind of project could we come up with that was really outside of the box—and outside of anything that I had been doing before? We ended up looking at performing a screen for novel genes in zebrafish and found many interesting ones involved in craniofacial development."

Bronner recruited postdoc Sean Megason, biologist Scott Fraser, and bioengineer Niles Pierce, and they pooled their disparate talents to create a new center for mapping vertebrate development—with Bronner bringing the developmental biology know-how, Megason and Fraser the tagging and imaging tools, and Pierce the molecular-detection methods he'd created in his lab. The ultimate goal? To create a "digital fish" to model the genetic orchestra that transforms an egg into an embryo.

The project was inspired by landmark studies on fruit flies conducted in the late 1970s and early 1980s by biologists Christiane Nüsslein-Volhard and Eric Wieschaus. Nüsslein-Volhard's and Wieschaus's research, which

Tatjana Sauka-Spengler (left) and Marcos Simoes-Costa prepare the reagents for a study of facial defects in zebrafish.

Tatiana Hochgreb (left) and Bronner at the centrifuge used for protein separations.

earned them a share of the Nobel Prize in Physiology or Medicine in 1995, was deceptively simple in plan: douse flies with chemical mutagens to muck up every developmentally interesting gene, and then see what happens.

"When they did this, they were considered sort of crazy, but it ended up being incredibly important and drove the entire field of *Drosophila* developmental biology for years," Bronner says. "So we thought we could do something similar in vertebrates, using zebrafish. Our plan was to do a screen to identify lots of developmentally important genes, and perhaps understand their function."

Instead of mutating genes, however, they decided to randomly tag proteins with green fluorescent protein—a common molecular marker that glows green under ultraviolet light and allows the tagged proteins to be spotted within specific cells, or even particular parts of cells.

To date, Bronner and her colleagues have produced some 260 green fluorescent zebrafish lines, which are housed in more than 800 clear acrylic tanks in a basement lab. Using those fish, she and her team have discovered several proteins that are crucial to fashioning a proper face.

For example, postdoc Tatiana Hochgreb has found a protein that seems to be involved in the transition from cartilage to bone in the jaw. In normal jaw development, cartilage cells build a scaffolding and then die, paving the way for bone-forming osteoblast cells to climb into the scaffolding and convert it to bone. The protein Hochgreb found, a DNA-binding protein, is made by a gene that turns on for only 24 hours or so between days three and four of embryonic development. But during that short interval, it tells the cartilage cells exactly when to die. That timing is critical: the cells *have* to die at a particular time, when the scaffolding is the right size. If not, the bone doesn't form correctly.

Moreover, Bronner says, "if you knock out this protein, you end up with extremely malformed jaws"—a fish "face" that is squished flat. The defects only affect the cranial bones, she says, "so some of the bones of the neck are still okay, and the trunk forms normally."

Intriguingly, many of these same proteins crop up in higher vertebrates, including us. Some may be associated with human craniofacial defects, like cleft palate. Tracing these proteins back to the network of genes that builds our faces could lead to new genetic screens, Bronner says, "so you'd know in advance that something is happening with a particular child." Not a bad day's work for a motley group of giant bloodsuckers and inoffensive, two-inch-long fish. E&S

Ruddock Professor of Biology Marianne Bronner has been at Caltech since 1996. She got her BS from Brown in 1975 and her PhD from Johns Hopkins in 1979; both degrees are in biophysics.

Scott Fraser is the Rosen Professor of Biology and professor of bioengineering.

Niles Pierce is a professor of applied and computational mathematics and bioengineering, as well as executive officer for bioengineering.

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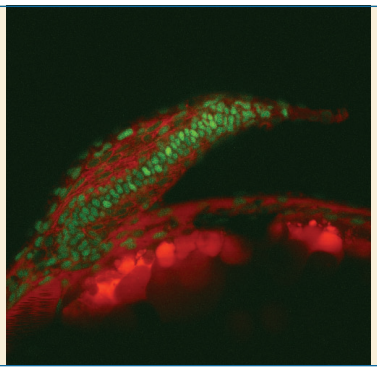

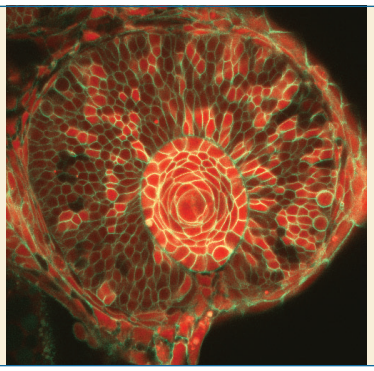

Fluorescently tagged proteins in a zebrafish's fin (far left) and eye (left). The tagging technique used in these pictures is the same as that used to identify proteins important for jaw formation.