Tales of a Travelin' Cell

by Douglas L. Smith
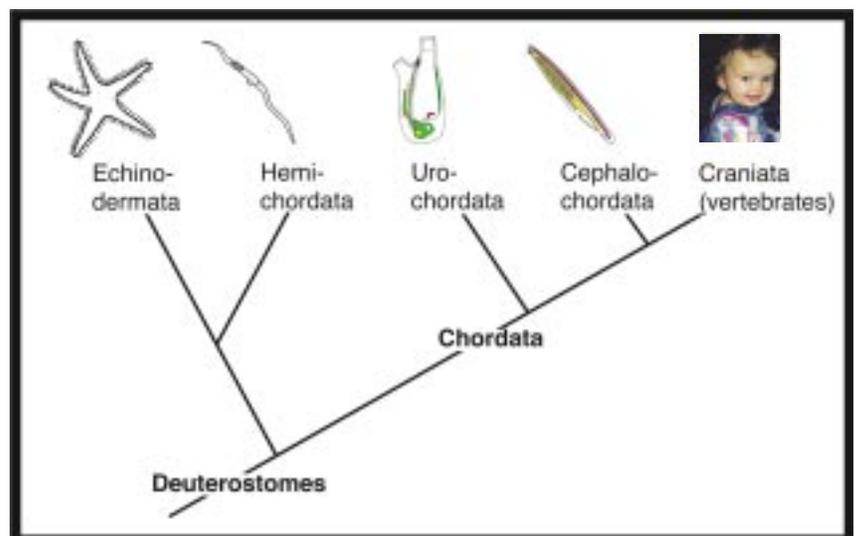
If Danny DeVito and Arnold Schwarzenegger can be twins, so too can the lamprey and the amphioxus—both are snakelike aquatic critters, but lampreys can be several feet long, while your average amphioxus tops out at a couple of inches. And if you looked at them when they were young, you'd swear they *were* twins. But the lampreys are vertebrates, albeit just barely—they are the simplest ones extant, and their backbones aren't even bone, but cartilage—and the amphioxus is the highest example of the next lowest branch of the chordate phylum. (The chordates are all animals with a hollow nerve cord down the back, regardless of whether they have a spinal column to keep it in.) The two species part paths as embryos when a group of cells called the neural crest appears in the lamprey, so Marianne Bronner-Fraser, the Ruddock Professor of Biology, studies those cells to address "two central questions in developmental biology: How do you build a complex adult organism from a single cell? And, how do species become different? Both are very difficult, very interesting questions."
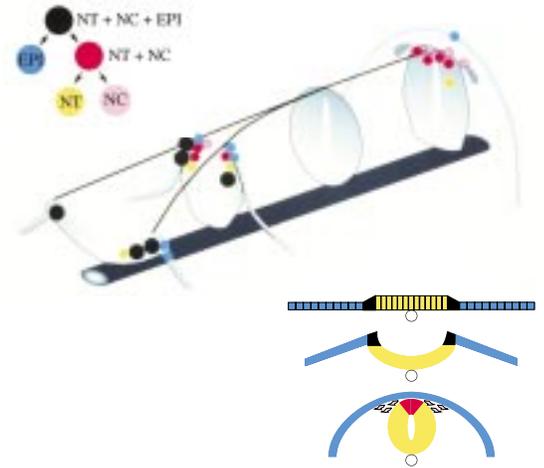
The neural crest cells emerge from the nascent central nervous system—the brain and spinal cord—shortly after its formation. Their progeny fan out through the embryo to become most of the rest of the nervous system. These cells also form most of the skull and jaw bones—if it weren't for the neural crest, we (and cats and dogs and crocodiles and frogs and pelicans) wouldn't have faces. But only the vertebrates have them. "The amphioxus is our closest living invertebrate relative," says Bronner-Fraser. "It has a very nice nerve cord similar to, but much more primitive than, our spinal cord. Its head has structures called gill arches that, in vertebrates, fill with neural crest cells and become the jaws and facial structures. It fascinates me that the two embryos resemble each other so closely, but that the amphioxus lacks this one particular cell type."

Every embryo starts out as a single cell—a fertilized egg—that divides repeatedly to form a hollow ball of identical cells. In animals, the ball grows what looks like a belly button (an innie),

Our branch of the tree of life. The Chordata, or chordates, include fish, amphibians, reptiles, birds, and mammals in the vertebrate class. The amphioxus is one of the Cephalochordata, and the Urochordata include the sea squirts.

and then something really amazing happens. The cells in the immediate vicinity of the navel plunge down through it to become a second layer of cells that expands to line the inside of the ball, like a balloon being inflated inside a bottle. This inner layer then subdivides to form a third layer, and the ball elongates to become a hollow cylinder. The outer layer is called the ectoderm, which will form the skin and the nervous system. The middle layer, the mesoderm, will become muscles and some organs, such as the heart and kidneys. The innermost layer, the endoderm, forms the viscera.

The neural crest cells, not surprisingly, come from the ectoderm. While the cells on the ecto-

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derm's outskirts (shown in blue in the inset above) will become skin, the cells in the central region thicken and change shape, morphing from sugar cubes into soda cans (yellow). The soda cans are called the neural plate, and this transformation marks the beginning of the nervous system. Like a rug being pushed up against a wall, the ecto-derm begins to fold up into two parallel ridges where the plate cells and skin cells meet, and the line of cells (black) on the crest of each ridge will become the neural crest cells. The flanking sheets of skin cells move toward each other as the neural plate sinks between them, until the neural plate

has been rolled up into the neural tube, which will become the brain and spinal cord. The tube zips itself shut from the head to the tail, with the neural fold cells being the teeth in the zipper. But they don't all remain teeth for long—as soon as the tube closes, some of them opt to become neural crest cells and promptly hit the road. "This one group of cells gives rise to cells that are as different as the bone cells in your face and the nerve cells in your guts," says Bronner-Fraser. "How they do it is a fascinating question, and really reflective of what's going on in the fertilized egg, where you have one cell that can give rise to all the cell types in the body. How does a cell decide whether to become a bone cell or a nerve cell? And how does it know where to go?"
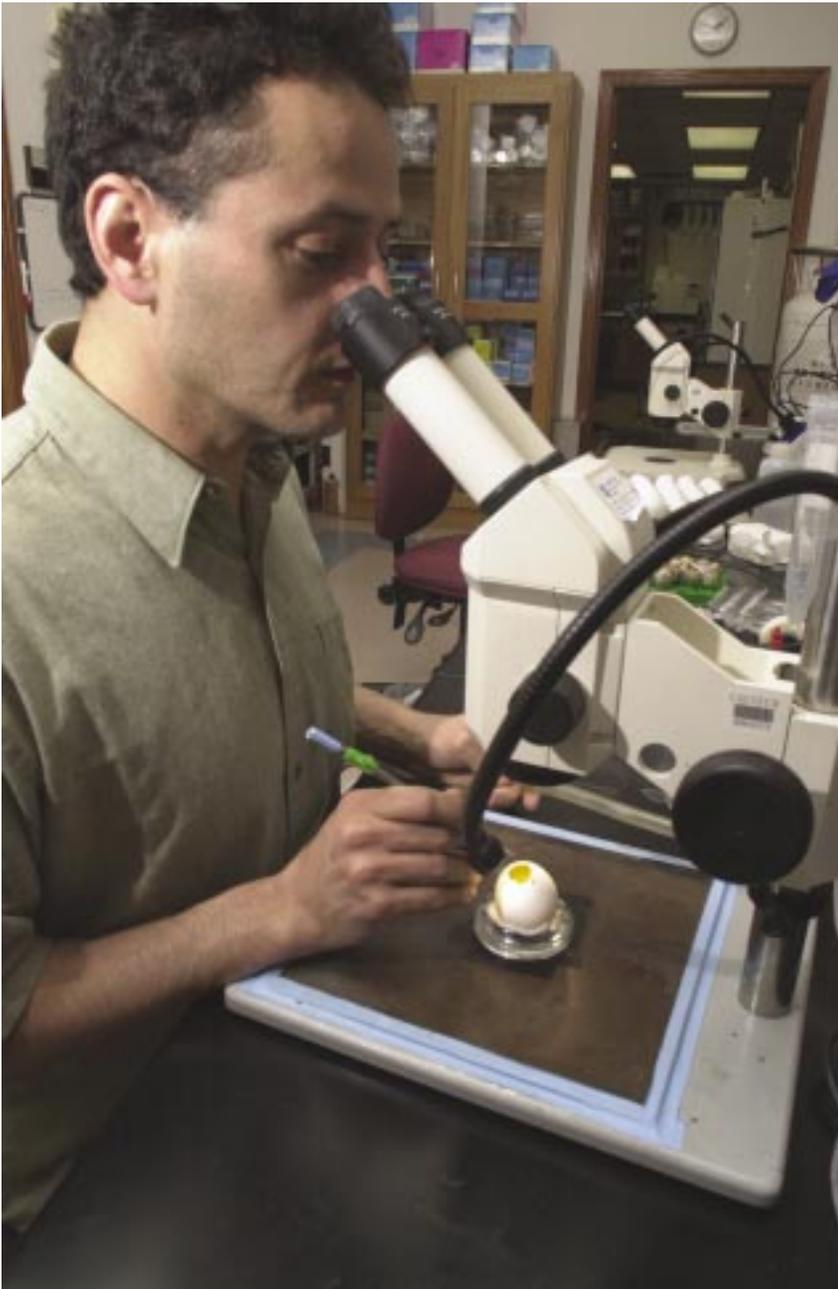
Or, for that matter, how does an ectoderm cell decide to become a neural crest cell in the first place? Since the cells always form at the boundary between the ectoderm and the neural plate, Bronner-Fraser wondered whether a process called induction was at work. Induction is "a kind of conversation between two tissues" that results in the creation of a new cell type, and is a staple of embryology. Signaling molecules from one tissue (the skin) bind to receptors in an adjoining tissue (the neural plate), turning on genes in the receiv-ing cells that transform them into a third tissue (the crest).

To find out whether induction was at work in the neural crest, postdoc Mark Selleck (now a professor at USC) put skin cells and neural plate cells together in a culture dish to see what would happen. And sure enough, neural crest cells formed at the junction. He repeated the experi-ment on chicken embryos, grafting little slivers of neural plate tissue under the skin in places it didn't belong. Says Bronner-Fraser, "We call this cut-and-paste biology. We do most of our work on chickens, because it's very easy to open up the egg, cut out bits of tissue, and move them around to different places. Then you can close the egg

same name, so to keep them straight, the genes' names are rendered in italics.

"There are basically two strategies available," says Bronner-Fraser. "We can try to discover a brand-new molecule. Or we can just look in the refrigerator and pull out all the molecules that are known to send similar signals in other situations, and see if they're doing the job here as well. It used to be that everyone was finding new molecules right and left. Every journal you'd go to, there'd be reports of proteins with weird names like 'sonic hedgehog,' or called by various initials—BMP, FGF, and so forth. We envisioned that there were thousands of signal paths, each with its own protein, and everyone would get to discover one. I think the most disappointing discovery in the last five years has been the fact that there may be only a small number of signaling molecules that get used over and over and over again. Now every time you look in a journal, you see the same protein doing something different. So maybe a signal that somebody else discovered helps form the ear is important here, too. We call this the 'usual suspects' approach."

However, no jury will convict unless the suspect is in the right place at the right time. One such protein, called Wnt (pronounced "wint"), was found by postdoc Martín García-Castro to hang out in skin cells. The Wnts are actually a family of closely related molecules that are the vertebrate equivalents of a fruit-fly signaling molecule called "wingless." (Three guesses what goes wrong when you tamper with it.) The people who found the vertebrate version called it "int," and when the fruit-fly connection was made, the "W" was added to the name.

But Wnt could be an innocent bystander—it might be doing something else, or nothing at all. So García-Castro tried blocking its signal to see if the neural crest cells would form without it. By flooding the cells with a Wnt inhibitor, he overloaded its receptors and basically crashed the

Above: This piece of neural plate tissue was laid on a piece of skin in a culture dish. Neural crest cells (dark stain) formed wherever the two tissues touched.

back up and the embryo will continue to develop very happily. So we can see if the cells will do something different if they're in a different place." But the result was the same—any time the two tissues met, they made neural crest cells. So the next step was to zero in on the responsible molecules.

Many signaling molecules are proteins, as are the receptor molecules, and a protein is manufactured in any given cell only when that cell's copy of the gene containing that protein's fabrication instructions is turned on. The interplay of protein and gene, stimulating the production of some proteins and inhibiting others, gives rise to the astonishing complexity of cells, and thus of life. Each protein and its corresponding gene share the

A gene need only be active at a very low level to be involved in a cellular

change, so its mRNA may only show up once or twice. In order to be sure of

finding such a needle, you have to pitch a ton of hay.

system. And behold, no neural crest cells.

Just one more piece of evidence was needed to nail down the case—was Wnt on its own sufficient to make neural crest cells? To find out, García-Castro added it to bits of neural plate tissue in a culture dish. The neural crest cells appeared and promptly set out for a stroll, just as if they were back home in the embryo. And to be sure he wasn't falling victim to cellular identity fraud, he checked their IDs with several molecular markers.

The fact that Wnt is both necessary and sufficient to make neural crest cells doesn't mean that it is the only thing involved in an actual organism. "This is just the tip of the iceberg," says Bronner-Fraser. "To generate a different cell type, all kinds

of changes must occur within that cell." Each of those changes is reflected by changes in the inventory of proteins found in the cell, each of which is the product of some gene. So postdoc Laura Gammill is trying to catalog the lot of them, using techniques developed for genomics.

Genomics, also called the "new biology," uses highly automated equipment to look at thousands of genes at a time and analyze their sequences or figure out their functions. In this case, Gammill collected all of the mRNA from some chicken embryos that were forming the neural crest. (Messenger RNA, or mRNA, is the molecule made by an activated gene that carries the gene's instructions to the cellular machinery.) She then made DNA copies of each piece of mRNA, and inserted each copy into a bacterium. Each bacterium was separated out into its own culture dish, and grown until its zillions of offspring had churned out usable amounts of that mRNA copy. Then a robot in the Genomics Technology Facility at the Beckman Institute daubed a little dollop from each dish onto hybridization filters, which are nylon membranes $8\frac{1}{2}$ inches square. Biologists call this a "macroarray," and they're not kidding— the Q-Bot can do 15 filters at a time (it takes several hours), and each one holds 18,432 spots. Gammill used eight filters, or 150,000 spots.
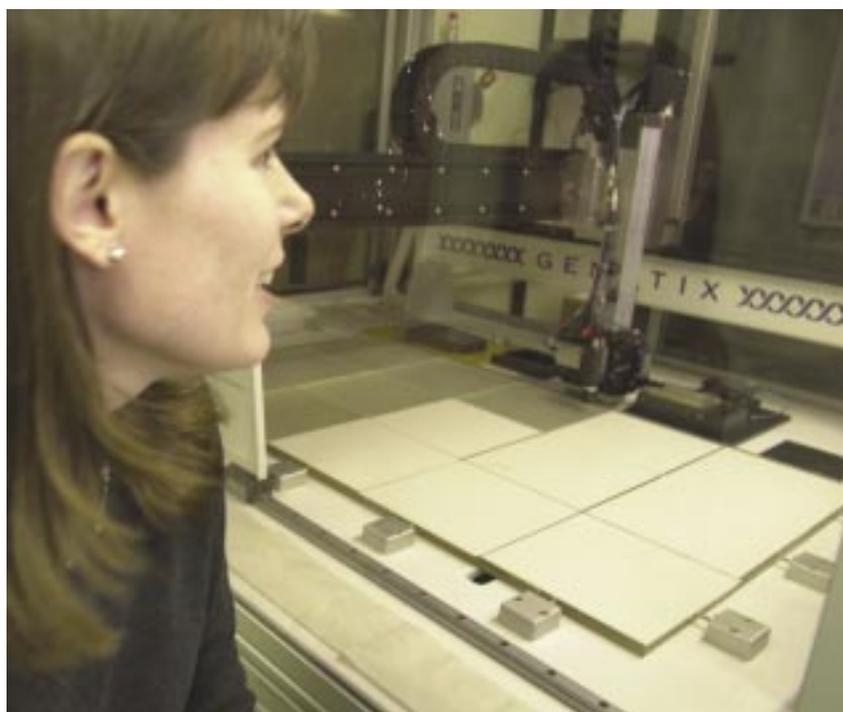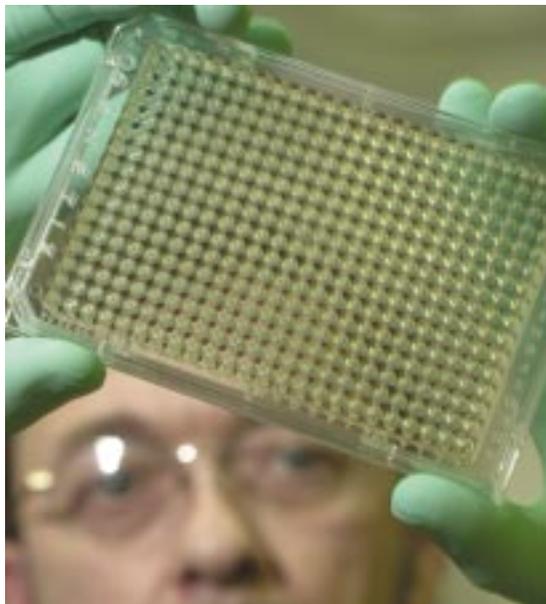
Only a few of these spots will hold anything interesting. You'll get several thousand spots containing a copy of a gene that does some vital but irrelevant thing like enabling the cell to divide, for example. That gene is turned on full blast, and the cell is cranking out its corresponding mRNA. But a gene need only be active at a very low level to be involved in a cellular change, so its mRNA may only show up once or twice. In order to be sure of finding such a needle, you have to pitch a ton of hay.

Thankfully, there's a way to toss out most of the hay. DNA is a double-stranded molecule, with the letters that contain the genetic informa-

Right: Neural crest cells (dark) are springing into being along this chicken embryo's neural tube, except for where a Wnt inhibitor was applied. The cells were made visible with a dye molecule attached to a DNA sequence that was designed to bind to the mRNA for *slug*, a gene known to be active in neural crest cells. Whatever cells have that gene turned on will produce that mRNA and take up the dye. Below: Adding Wnt to neural plate tissue in a culture dish (right) was like firing a starter's pistol: a whole bunch of cells suddenly took off for parts unknown. No Wnt, no wanderlust (left); the cells remained neural plate cells and stayed home. Both samples were stained with HNK-1, a non-specific agent that binds to many tissue types.
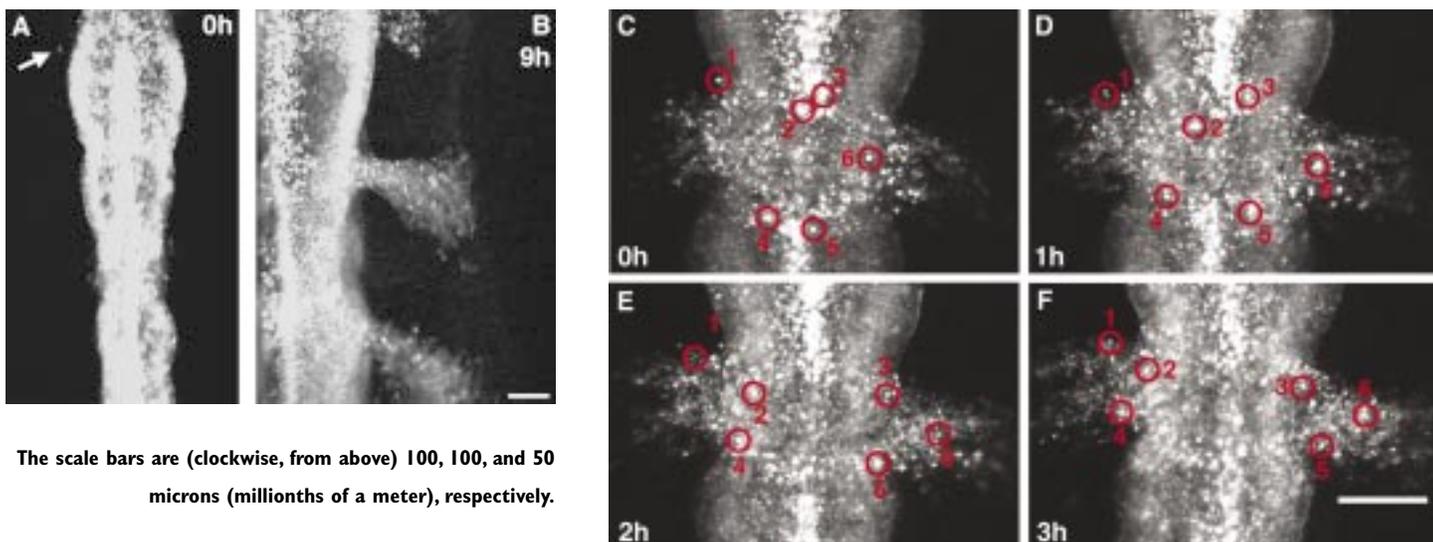
tion arrayed in sequence along one strand. Each letter on this strand will bind only to its complementary letter on the other strand—As with Ts, and Cs with Gs. So Gammill sandwiched some skin and neural plate tissue together to induce neural crest cell formation, extracted the tissues' mRNAs, and made single-stranded DNA copies of them. Then she made a separate pool of single-stranded DNA molecules whose letters were complementary to mRNAs she had taken from skin cells and neural plate cells that were not in contact with each other. Any gene active in both the neural crest cells and the skin or neural plate cells—the common, uninteresting genes—would have a DNA strand in each pool. Stirring the two together allowed each such strand to find and bind to its mate. This double-stranded DNA was separated out, leaving the single-stranded neural crest DNA, which she then used to search for spots of matching mRNA on the filters.

Says Bronner-Fraser, "We went into this thinking, 'Well, when you generate a new cell type, what would you expect? Are 1,000 genes involved in this process, or 2,000?' We had no idea what the scale would be." She now thinks there are considerably fewer genes involved, but nobody really knows for sure.

Gammill has found about 100 genes so far, which she is running through a database to see if they resemble ones of known function. She comes up empty about 10 percent of the time, but many of the ones she's identified fall into groups related to cell proliferation and locomotion. Says Bronner-Fraser, "It's as if these cells are being generated so that they can quickly become migratory cells, which makes sense in hindsight, because the first thing they do is leave the neural tube." In other words, they're born with their knapsacks packed. They're good to go.

These itinerant cells thrust out long filipods—literally "thread feet"—but not much is known about how they propel themselves. Says Bronner-Fraser, "The classic way to study how cells move is to put fibroblast cells—a type of cell found in connective tissue—in a flat dish, which probably doesn't relate really well to how cells move in embryos." Some of the genes Gammill found make proteins in the cytoskeleton, which is the scaffolding within a cell that gives it its shape. Changing from a regimented, immobile brick in a wall of tissue to a freewheeling, self-propelled blob in a lava lamp "obviously requires changes in the underlying structure of the cell, but it starts happening much earlier than we realized," Gammill says. Other genes she found encode proteins in the extracellular matrix, which is the gelatinous goop outside the cells that the neural crest cells grab onto in order to pull themselves along. "We do know that if you block some of these interactions, the cells don't do as well, but a lot of times they still get to the end point," Bronner-Fraser says. "So what makes them motile
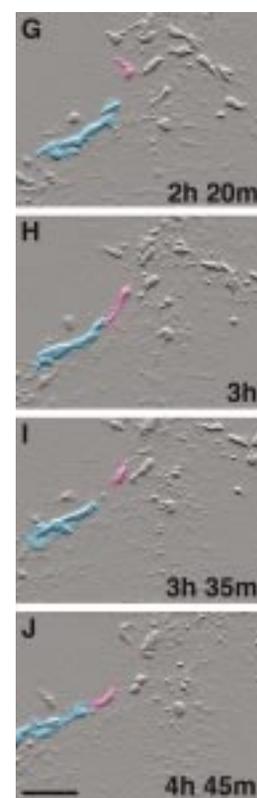
The scale bars are (clockwise, from above) 100, 100, and 50 microns (millionths of a meter), respectively.

is a really big question. I'm very interested in it, but I don't quite know how to get at it yet."

How they find their way is another good question. Bronner-Fraser is collaborating on that one with her hyphenate, Scott Fraser, the Rosen Professor of Biology and principal investigator at the Beckman Institute's Biological Imaging Resource Center, who makes movies of embryonic cells on the march. (In fact, his lab has developed a whole set of techniques for watching cells go about their business deep within opaque embryos, but that's another article.) Any individual cell can be tracked for as long as it remains on screen, and if you watch a lot of these movies, you'll notice that one cell frequently leads the way, making a path that the others follow. Says Fraser, "Sometimes they follow one another in a polite, well-behaved line—in what we call the 'English queue' mode—and sometimes they just climb and claw all over one another to get to the front of the line. We call that the 'professional wrestler' mode." Some of the Fraser lab's movies are on the Web at http://bioimaging.caltech.edu/neuralcrestpk.html; try movies 3 and 4. Says Bronner-Fraser, "Often, you'll see cells link to one another as if they're communicating. We didn't know they did that before." This leads to a whole new set of questions: What happens if you wipe out the leader? Will the next cell in line still know where to go? Will a new leader step in? "We went in thinking, 'These cells are smart. They always go to the right places.' Well, it turns out that a lot of the cues that keep them on track are not things that are saying, 'Hey, come this way,' but things saying, 'Stay away!'"

For example, postdoc Cathy Krull in Bronner-Fraser's lab (now a professor at the University of Missouri) and Rusty Lansford, a senior research fellow in Fraser's lab, have discovered that an inhibitor molecule called ephrin funnels the outgoing neural crest cells onto a set of prescribed paths as they exit the neural tube. Without ephrin

In these photo sequences from the Fraser lab, the neural crest cells are bright against the background tissue. What starts with a single neural crest cell (arrowed) in A becomes a mass exodus nine hours later in B. But even then you can track the cell(s) of your choice, as shown by the numbered circles in C through F. In G through J, the cells have been "embossed" to make them easier to see. As a column of cells (blue) marches off, a nearby cell (magenta) reaches out to touch the last cell in line, and then joins the parade.

(or with an overdose of it), the neural crest cells overrun the surrounding tissue like a horde of lemmings. And Bronner-Fraser postdoc Maria Elena de Bellard has found that another inhibitor, called slit, keeps the wrong set of neural crest cells out of the viscera so that the right ones can enter and become the autonomic nerves in charge of your digestive tract. It now appears that neural crest cells can go anywhere they please unless they are specifically excluded, and, left to their own devices, they go wandering willy-nilly and wind up in all the wrong places. So they aren't smart cells that know where to go, but dumb cells that react to their environments.

Which may explain what happens when neural crest cells turn bad. "Many invasive cancers arise from cells of a neural-crest origin," says Bronner-Fraser. For example, some neural crest cells form the pigment cells, or melanocytes, in your skin. When they run amok, you get melanoma, which is a malignant cancer with a nasty tendency to spread. And there's neurofibromatosis, which some people think is what the so-called Elephant Man had. "An amazing array of tumors arise from these cells, and the fact that they are so migratory in the embryo makes you wonder if this contributes to their ability to become metastatic later. Some labs are looking at neural-crest-derived tumors and comparing their properties to neural crest cells, and it turns out that the worst tumors are the ones that most closely resemble the embryonic cells. We're doing the basic science on the neural crest cells, providing a point of comparison." Perhaps one reason for those cancers' invasiveness is that the molecular "Keep Out!" signs aren't present in adults. "More happens than that. Tumor cells can actually break down barriers that they may not have been able to as embryonic cells, so they're like superembryonic cells. But the fact that the environment is not embryonic probably contributes."

When the cell *is* in its proper environment, Bronner-Fraser says, "it's very heartening that we're finding a manageable number of genes, because we can now go back and test the function of the most interesting ones using classical bio-

logical techniques." In other words, the researchers can inject the gene back into the embryo and see what it does, or block the protein's receptors and see what goes wrong, just as García-Castro did with Wnt. Or they can use various methods to turn the gene on and off and see what happens, like flicking a light switch to see which outlet it controls. And the gene array on a filter can be reused indefinitely, so you can go back to that filter at any time to try something new. Then, when you find a gene that intrigues you, you can go to the corresponding culture dish, where you now have it on tap.
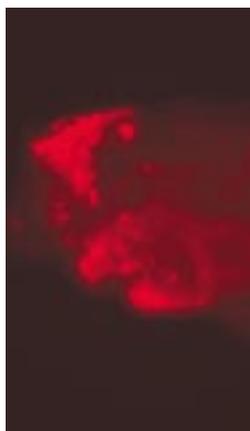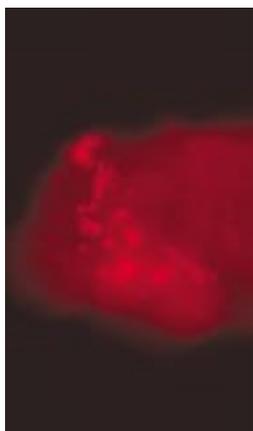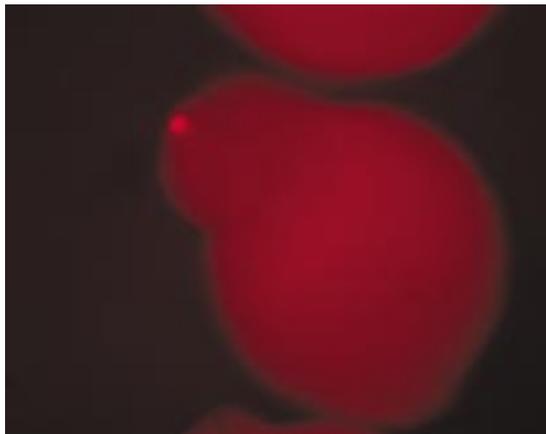
Bronner-Fraser plans to make a series of genomic snapshots spanning the migration process. "We've only looked at the cells as they're getting ready to migrate, but we can ask what happens when they're actively migrating. Or when they're about to stop migrating—that is, how do they know when they've arrived? Maybe the stop receptors are missing on cancer cells. Genomics is an incredible tool that lets us explore the system in a much more complex way than we were able to before. It's very exciting because we can get answers that just weren't available to us even two years ago. So it's a great time to be in biology."

Beyond following the neural crest cells' life cycle, Bronner-Fraser's lab is trying to answer the larger question of how these cells evolved in the first place. This involves looking at the lamprey, which has them, and the amphioxus, which doesn't. Lampreys are the vampires of the high seas—they attach themselves to fish with their suction-cup-like mouths and drink their fill of blood before moving on. Says Bronner-Fraser, "They're slimy and they're horrible and they don't have much of a forebrain and they have this circular mouth, with teeth all around the edges. I wouldn't want to swim with them." But they do have one redeeming quality. "I gave a departmental seminar once," she recalls, "and Seymour Benzer [Boswell Professor of Neuroscience,
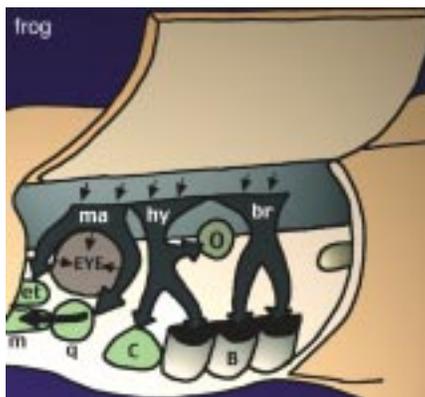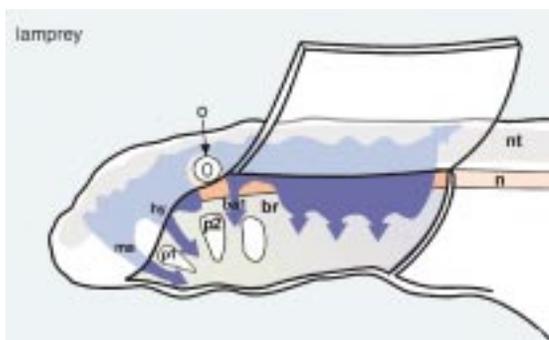
Right: In the developmental-biology equivalent of putting radio collars on cells, McCauley injected a dye called DiI into the neural-crest-forming region in the back of the head of a lamprey embryo in the early stages of neural-crest-cell migration (top). Two days' time (bottom left) finds the cells en route to the mouth, which is at the bottom of the picture. Six days after injection (bottom right), some cells have reached their destination—the upper and lower lips, which they fill like a hand in a sock puppet. In higher vertebrates, these cells would become the jaws.

Below: The neural-crest-cell migration routes in lamprey and frog embryos. Both have mandibular (ma), hyoid (hy) and branchial (br) streams, so named for their destinations. The first two streams become facial cartilage in the lamprey and facial bones (green) in the frog. In both species, the hyoid and branchial streams surround the otic vesicle (O), which becomes the ear canal. The branchial streams become the branchial arches, a subset of the gill arches. (The "ba1" stream in the lamprey goes to the first, or headmost, branchial arch, labeled "B" in the frog.) Pouches of tissue, two of which are labeled "p1" and "p2" in the lamprey, lie between the gill arches. The neural tube is marked "nt," and "n" is the notochord, a stiff rod of cells found in all chordate embryos that acts as the backbone. Lamprey drawing by David McCauley; frog drawing by Senior Research Fellow Carole LaBonne after Roberto Mayor et al. "Development of Neural Crest in *Xenopus*," *Current Topics in Developmental Biology*, vol. 43, 1999, Academic Press.
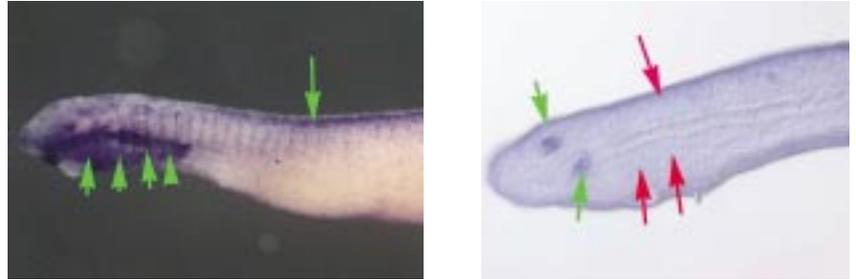

Emeritus] got really excited. He said, 'I didn't know you worked with lampreys. Did you know that they're a delicacy?' It turns out that in Portugal they actually eat these things." Benzer even sent her some recipes, which she hasn't yet had the nerve to try.

Lampreys spawn in cold freshwater streams, and in the early 20th century they invaded the upper Great Lakes, where they've been wreaking havoc ever since. So postdoc David McCauley spends every June at the Great Lakes Fishery Commission's research station at Hammond Bay on the shores of Lake Huron. Says McCauley, "Their mission is to control the lamprey, so it's kind of odd—I'm trying to figure out how to raise them, while they're trying to kill them." Collecting lampreys is easy, he says. "The males build nests by moving rocks on the streambeds with their mouths—it's amazing how big a rock they can move—so you just walk down the middle of the stream and you'll come upon three or four lampreys in this shallow depression, the spawning nest, and you just grab 'em." He shucks all the eggs and sperm into one beaker—it's sort of like milking cows—swirls the beaker a few times and lets it sit for about 15 minutes. The fertilized eggs are about a millimeter in diameter, opaque, white, and yolky, so they're not the easiest things to work with. "But the nice thing is, they develop very slowly," says Bronner-Fraser. "Most organisms studied in developmental labs—zebra fish, for example—develop really fast. So if you're interested in a particular stage, you might have to stay up all night to get to the stage you want. These guys develop so slowly that you could sleep for 12 hours and come back and still be all right."

The progeny of neural crest cells

The green arrows show the different staining patterns for *ap2* in the lamprey (left) and amphioxus (right). In the lamprey, *ap2* appears in the neural tube (top) and gill arches (bottom). In the amphioxus, these structures are marked with red arrows, and *ap2* is found instead in the brain (upper arrow) and the pre-oral pit.

Wading for amphioxuses in Florida. Linda Holland is second from right, with the white eyeshade.

from different places along the neural tube wind up in different parts of the body, so McCauley traced their migration routes by injecting a dye called DiI into various locations down the length of the tube. DiI gets soaked up by the cell membrane from the extracellular fluid, so you can just squirt a tiny droplet under the skin—much easier than trying to get a hypodermic needle into an individual cell! Similar experiments on frog embryos had been done by Andres Collazo (now at the House Ear Institute) when he was a postdoc with Scott Fraser and, says McCauley, "the way lamprey cells migrate is not so different. The structures they give rise to are different—lampreys have no jaws, so lamprey neural crest cells give rise to cartilage in the branchial arches instead—but there are similar populations of cells going to

similar places. They just do different things when they get there." In other words, there's no gradual transition to neural-crest-cell-ness. The crest cells in the lowliest vertebrate have all the attributes of those in more sophisticated creatures.

So what happened between the amphioxus and the lamprey? Does the amphioxus also have the neural-crest-forming genes, and, if so, what are they doing? Grad student Daniel Meulemans found that a gene called *ap2*, which is essential to the formation of the neural-crest-derived facial bones and nerves, also shows up in the amphioxus. In fact, in the early stages of development, *ap2* does the same thing in both species—it's turned on in the epidermal cells that will become skin, but not those that will become the neural plate. Then it turns off for a while, and when it turns back on again, its role has changed. It shows up in the amphioxus's cerebral vesicle, which is what passes for a brain, and in the pre-oral pit, says Meulemans, "which is a weird gland that may be the amphioxus version of the pituitary."

"We found this to be true for almost everything that we looked at," says Bronner-Fraser. "It was as if the amphioxus had the whole array of genes that the vertebrates had, but they weren't using them in the same way." In order to make their new tissues, the vertebrates apparently co-opted existing genes and gave them new duties instead of creating new genes from scratch.

Just as lampreys draw McCauley to Lake Huron, Meulemans spends his summers in the wilds of Tampa, Florida, in search of his quarry. Adult amphioxuses live in shallow seawater, where they carpet the bottom, anchoring themselves in the mud by their tails and straining plankton from the murky water. To collect them, you need to filter feed as well—teams of people wade into chest-deep water armed with long-handled shovels, archaeologist-style sieves, and buckets. You shovel the mud into the sieve, and pick out the amphioxuses. "Tampa is the best place to

In 50 days, an amphioxus grows from a ball of cells to a mature couch potato, buried in mud up to its neck. Like a guy with an empty popcorn bowl, it gets up long enough to feed itself, rising to the tailward dashed line. It will even bestir itself enough to swim to a new spot now and then.

Figures by Nancy J. Haver from pp. 370 and 371 of *Embryology: Constructing the Organism*, edited by Scott F. Gilbert and Anne M. Raunio, Sinauer Associates, 1997.

collect them in the U.S. because their population density is so high, and Nick and Linda Holland have worked out the ecology and know just where to get them," says Meulemans. "It's a very cool event—the whole U.S. and European amphioxus community shows up to harvest them."

The Hollands, who are at the Scripps Institution of Oceanography in La Jolla, are the deans of amphioxus research, and have developed an electro-stimulation method to induce the amphioxuses to spawn on cue. Still, the technique only works during breeding time—at night during the summer. Says Meulemans, "Traditionally, you'd collect them every day, then zap them in the evening and hope they're in the mood. They only spawn once, so if they do, you throw them back into the ocean. And if they aren't in the mood, you try them again the next night." But last year, Meulemans discovered that they would continue to spawn indefinitely every two weeks if he put lights over their tanks on a cycle that mimicked a midsummer's day. "So they do have potential as a lab animal, but it's hard to keep the plankton levels high enough for them to spawn without the nutrients polluting the tank at the same time. That's why they normally only spawn in the summer—there's lots of plankton."

The neural crest cells' reassigned genes are the first tool of war for the vertebrates. "The amphioxus doesn't have that, so all it can do is sit around and filter feed. It retains its pacifist lifestyle."

(Lampreys are easier to raise in the lab, but like the salmon they prey on, once they spawn, they die. So the trick is to make them chill out. Literally. "Spawning is temperature-induced," says McCauley. "So if you keep them very cold—

5° C—they won't spawn. Their metabolism shuts down, and you can hold them in tanks for as long as you like." Then, when you're ready for an experiment, you just plop them into 15° water and you're off to the races.)

Amphioxus adults and lamprey larvae are both filter-feeding bottom-dwellers. Says Meulemans, "The crest-cell derivatives become important when the animal becomes predatory. They go into making things like the jaws, the muscular pharynx, and a better peripheral and sensory nervous system for faster movement and heightened senses." In other words, the neural crest cells' reassigned genes are the first tool of war for the vertebrates. "The amphioxus doesn't have that, so all it can do is sit around and filter feed. It retains its pacifist lifestyle."

The thing that allows the lamprey (and the rest of us vertebrates) to beat our gill arches into swords is a stretch of DNA called the *cis*-regulatory region, which lies just ahead of each gene. (See *E&S*, 2001, No. 3/4.) The *cis*-regulatory region contains binding sites for assorted signaling molecules that appear in various combinations in different parts of the embryo at different times and the sum of whose effects turns the gene on or off.

To try to understand how the genes' functions shifted, Meulemans took the *cis*-regulatory region from an amphioxus gene and coupled it to the DNA for a protein that caused the tissue to turn blue wherever the gene was turned on—what's called a "reporter gene" in the trade. He then injected this homemade gene into chicken embryos and applied an electric current across the neural tube in a process called electroporation. The current temporarily opened pores in the cell membranes, letting the DNA in. And because the DNA has a negative charge, it got pulled toward the positive electrode. So the new gene only entered the embryo's crest cells on one side of the tube, while the other side, with its original patrimony of chicken genes, served as the control.

The gene whose *cis*-regulatory region Meulemans borrowed is called *snail*, and it is one of the first neural crest genes turned on. In vertebrates, it's active in the neural-crest-forming region and in the peripatetic neural crest cells. In the amphioxus, it's active throughout the nervous system, not just where the neural crest cells would form, and it's not turned on in any migratory cells whatsoever. Meulemans found that the reporter gene turned on as it would in the amphioxus, confirming that evolution had changed how the gene is used.

So the question now is, what changed in the *cis*-regulatory region? Amphioxus eggs are barely visible to the naked eye and are notoriously hard to work with, but this past summer, the Hollands succeeded in injecting them with a reporter gene that Meulemans had constructed—the first time that foreign DNA had ever been put into an amphioxus embryo. This means that genes from either organism can now be put into the other one, and the problem can be attacked from both sides.

Says Bronner-Fraser, "Evolution is a hard question, because we don't have the ancestors around to look at—we can only look at the organisms that are still alive and try to extrapolate backward. We tend to be very vertebrate-centric as human beings, so we know a lot about a few vertebrates, but vertebrates represent a very small portion of the evolutionary tree. Very little is known about many of the creepy-crawly organisms, but it's really critical to study a lot of different organisms in order to understand their relationships. For example, jellyfish have muscles, but they don't have the layer of cells called the mesoderm that, in most other organisms, muscle comes from. So a lot of people are trying to figure out how that change occurred. Our ability at Caltech to make arrays of all the genes that are turned on at a particular place at a particular time, and then subtract away the common genes, is really exciting. I've been working on this one problem all my adult life—the core problem hasn't changed, it's just that the way we get at it has changed. It's hard to say what I'm going to be doing in five years, because each thing you do changes the next thing. That's why I love science." □

Marianne Bronner-Fraser has been at Caltech since 1996. She got her Sc.B. from Brown in 1975 and her Ph.D. from Johns Hopkins in 1979; both are in biophysics. She is on the NASA Life Sciences Panel for Developmental Biology, has chaired the Gordon Conference on Neural Development, and has been Caltech's faculty chair since 2001.

Two Lamprey Recipes

Cleaning and Marinating the Lamprey (Not for the weak-stomached!):

Immerse the lamprey in hot water. Remove the mucus by scraping the skin with a knife; finish by rubbing with a piece of cloth. Cut off and discard the tail (about six inches). Hang the lamprey by the head over a bowl containing a tablespoon of vinegar. Open the branchial-holes region to allow the blood to drip out, and wash the lamprey with seven ounces of red wine. Stir the blood-wine mixture as needed to avoid coagulation; set aside. Eviscerate the lamprey and remove the notochord, which is dark in color and lies along the back wall of the abdominal cavity. Prepare to remove the head by making a surficial cut all the way around the body. Pull off the head and be sure to get the large piece of associated cartilage. Discard the head and the cartilage. Wash again. Cut the lamprey into $2^1/_2$-inch pieces. Marinate the pieces for at least two hours (five or more is better) with salt, pepper, wine, bay leaf, parsley, and cut-up carrots and onions.

Lamprey "Bordeaux style" (Lampreia Bordalesa):

Heat the lamprey, marinade and all, with butter and crushed garlic in a pan until everything turns color. Add white wine and some fish soup, season and cover. Once the lamprey is cooked, remove the lamprey pieces. Return the sauce to the boil until well cooked. Turn down the heat and add the blood and some lemon juice. Let simmer without boiling. Serve the lamprey and the sauce atop thin pieces of toasted bread. Serve with white rice.

Lamprey Rice (Arroz de Lampreia):

Brown the lamprey pieces in a pan with about six tablespoons of olive oil, crushed garlic, salt, and pepper. Add small portions of water, enough to make one quart of sauce. Let boil for an hour. Taste and adjust seasonings. Add one pound of rice and the lamprey blood. Reurn to boil, then cook slowly until the rice is done. The sauce should remain very liquid. Serve piping hot.

Adapted from translations by Paulo Vaz-Pires from *A Cozinha Ideal* (Ideal Cooking), 9th edition, Manuel Ferreira, Domingos Barreira, Lisbon, Portugal, 1988; and from *Tesouro das Cozinheiras* (Treasures of the Chef), Mirene, Porto Editora, Porto, Portugal, 1993. The translations appeared in *Seiche*, the newsletter of the University of Minnesota Sea Grant Program, in April 1996.