velopment where they help to regulate the growth of embryonic connective tissues.

In the adult, TGF-β assists in healing wounds by luring immune cells to injury sites to destroy invading pathogens and remove dead tissue. TGF-β also triggers production of new connective tissue proteins, such as collagen and fibronectin, that help to close wounds. Buoyed by these observations, plus the results of numerous animal studies that have shown that a single topical dose of TGF-β enhances wound healing, the biotechnology industry has started testing the efficacy of TGF-β to aid wound healing in humans.

TGF-β has already shown promise in preliminary trials in patients suffering from a type of retinal deterioration called a macular hole. This condition, in which a hole appears in the macula, the central portion of the retina, costs some 20,000 elderly Americans their sight each year. Macular holes can be treated with surgery to reattach the retina to the underlying epithelium, but the operation fails to seal the macular holes nearly half of the time. Judging by the small clinical trials completed so far, this result can be improved upon by administering TGF-β into the eye at the time the surgery is performed, says John Thompson of St. Joseph Hospital in Baltimore, who helped develop the technique. "We can close about 95% of holes using TGF-β, compared to about 53% without it," he says.

Celtrix Pharmaceuticals, a biotech firm in Santa Clara that makes TGF-β, is now running a much larger trial, including 120 patients, to see if these promising early results will be borne out. Celtrix is also one of several biotech companies testing the ability of TGF-β and other growth factors, such as platelet-derived growth factor, to heal the skin ulcers that plague people with diabetes and circulatory diseases.

Tissue repair in the adult "is nothing but a recapitulation of what goes on in the embryo."

—Michael Sporn

All these trials involve giving growth factors to speed healing, but paradoxically, in some circumstances it may actually be better to inhibit TGF-β's activity. And those circumstances again demonstrate the advantages of being able to mimic embryonic processes.

A wound to the fetus in the womb heals without a scar. The reason may be, says Sporn, that fetal wounds contain little TGF-β, which can contribute to scar formation by stimulating the growth of connective tissue. That observation leads to the possibility that antibodies to TGF-β might one day be used to mop up excessive amounts of the protein in conditions where scarring is disfiguring or debilitating—for example, following severe burns, or for people suffering from glomerulonephritis, a condition in which the membranes of the kidneys may become so scarred that the kidneys fail.

TGF-β isn't the only type of growth factor that the biotech industry is concentrating on in an effort to mimic what happens to the developing embryo. The others include the bone-growth–promoting factors—called either osteogenic or bone morphogenic proteins—which help model the embryonic skeleton and allow adult bone to remold itself in response to changing stresses; these might be used for healing bone fractures (*Science*, 21 January, p. 324). For example, Creative BioMolecules in Hopkinton, Massachusetts, in partnership with a biotech subsidiary of the orthopedic product company Stryker Corporation of Kalamazoo, Michigan, is in the final stages of clinical trials testing the ability of osteogenic proteins to mend difficult-to-heal fractures of the tibia, the major weight-bearing bone of the lower leg. "Each year in the United States about 40 to 50 thousand people suffer complicated fractures that, because of infections or other problems, fail to heal," says Marc Charette, senior director of research and development at Creative BioMolecules. Usually, the only option is a bone graft from the patient's own hip or a bone transplant from a cadaver, which is risky because it can trigger a rejection response or carry infections. "A nice alternative would be to be able to go to the shelf and take a bottle of [osteogenic proteins] and apply it as paste to the fracture," says Charette.

Other possibilities being explored for clinical use include the neurotrophic factors—proteins such as nerve growth factor, insulin-like growth factor, and ciliary neurotrophic factor (CNTF)—that help neurons grow in the developing embryo and ensure their survival in the adult. Now, a battery of biotech companies is intent on developing them as drugs to treat neurodegenerative diseases, including Alzheimer's, Parkinson's, amyotrophic lateral sclerosis (ALS) or Lou Gehrig's disease, and peripheral nerve degeneration. Successful clinical development of these factors may not be easy, however. One indication comes from a trial testing CNTF in ALS patients, which was canceled by Regeneron Pharmaceuticals, the company conducting it, after the factor failed to help the patients (*Science*, 6 May, p. 772). Still, given that the diseases for which the new therapies are wanted are for the most part untreatable, efforts to find out whether these or as-yet-undiscovered growth and differentiation factors can help the patients will continue.

–Rachel Nowak

AXON GUIDANCE

Wiring the Nervous System

Navigating the interstates from New York to San Francisco is a tough job for a driver—but it's easy compared to what the neurons of the developing nervous system must do to reach their goals. During embryogenesis, more than a hundred billion fledgling neurons shoot out slender fibers known as axons that ultimately form an estimated hundred trillion links with their target cells. And not only do these axons often have to travel a long way—from the muscles of the foot or hand all the way to the brain, for example—they must also find precisely the right cells to hook up with at their destinations. "How are axons able to grow through the embryonic environment with unerring precision?" wonders Marc Tessier-Lavigne, a Howard Hughes Medical Institute (HHMI) investigator at the University of California (UC), San Francisco.

Though that question has intrigued developmental biologists for more than a century, only recently have they begun to get a handle on the road signs that guide the roving axons to their journey's end. They have found that an axon is steered by an array of guidance molecules in different locations: fixed on the surfaces of cells, within the extracellular matrix (a meshlike structure that fills in the space between cells), or secreted by the axon's targets.

The swiftness with which these discoveries are being made has accelerated in recent months. The first class of diffusible axon chemoattractant molecules was reported in August in *Cell*, when Tessier-Lavigne and his colleagues announced the discovery in chick brains of proteins called netrins, which are thought to steer axons around developing spinal cords. And to the delight of those in the field, efforts in vertebrates are dovetailing with work on the simpler nervous systems of worms and fruit flies.

"The pace has picked up quickly. The molecules that are being found are just the tip of the iceberg," says Corey Goodman, an HHMI investigator at UC Berkeley. Some researchers are even tantalized by the prospect that guidance molecules might help damaged or severed nerves regrow correctly, although they caution that much more work will be needed to establish that. "Everyone thinks there's going to be interesting clinical applications, but that won't happen for a number of years," says Goodman.

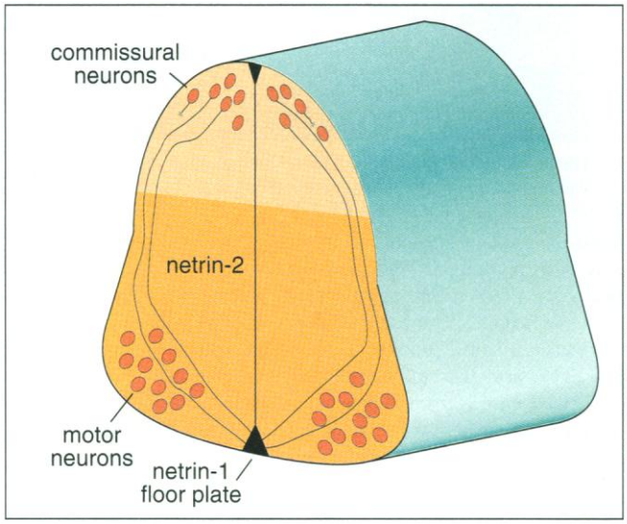
The first investigations into axon guidance emerged at the turn of the century in the work of eminent Spanish neurobiologist Santiago Ramón y Cajal. He not only noted that one neuron connects with another by sending out a single fiber, the axon, but also discovered that an extending axon has on its tip an amoebalike "growth cone." This cone leads the way as the axon tunnels through tissues and skirts obstacles until it reaches its target. Once there, the growth cone metamorphoses into a presynaptic terminal, a specialized structure that allows neurons to exchange signals.

Cajal's scents. In 1892, Ramón y Cajal put forth the notion that growth cones are like immune cells known as leukocytes, which sniff out chemical "scents" released by different tissues. This prescient suggestion lingered, although researchers made little progress in identifying diffusible molecules that could attract growth cones until the late 1970s. Instead, much work focused on cell-surface proteins such as cell adhesion molecules, which help cells attach to one another and migrate over each other. But then the idea of long-range chemotropism came roaring back to life, particularly in 1979 when Ross Gunderson and John Barrett at the University of Miami School of Medicine reported that the growth cones of certain axons would, in the petri dish, follow gradients of a protein known as nerve growth factor (NGF).

Over time, however, the promise of NGF as an axon guidance molecule has faded. No one has yet been able to establish that the protein plays that role in any developing organism. But in spite of that, the NGF discovery brought a surge of fresh interest into the search for the chemical cues for axon guidance—and out of that interest came two major developments. The first occurred in 1983, when Andrew Lumsden, now at Guys Hospital in London, and Alun Davies, now at St. Andrew College in Scotland, developed a novel assay for axon chemoattraction. The two affixed selections of axons and various tissue samples to collagen gels, which allow secreted molecules to diffuse, while keeping the neurons and other cells separated. In certain combinations, the axons' growth cones would advance across the gel towards the tissue sample and even change directions to find it. This new assay "blew the field open. It showed that a target could release a diffusible agent that promoted its own innervation," recalls Dennis O'Leary of the Salk Institute.

The second major advance was the development of another research tool: the growth cone collapse assay, created by Jonathan Raper and Josef Kapfhammer of the Max Planck Institute for Developmental Biology in Tübingen, Germany. This new tool effectively launched the search for molecules that provide not attractive, but inhibitory or repulsive cues for the navigating axons.

Even as the story of chemoattraction was emerging in the 1980s, hints of these other, negative guidance mechanisms had begun to appear. In addition to cell adhesion molecules and diffusible chemoattractants, an axon's journey seemed to be influenced by a host of contact-mediated repulsive or inhibitory cues provided by molecules within the extracellular matrix or on the surface of cells, including other axons. Raper and Kapfhammer were the first to demonstrate that, if the growth cone of one specific kind of axon came into contact with certain other kinds of axons, it would collapse, temporarily paralyzing the axon until the growth cone re-formed and went off in another direction. In 1990, they developed a test that allowed researchers to mix tissue extracts with growing axons and discern whether the growth cones collapsed.



MARC TESSIER-LAVIGNE/UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Guided tour. Produced in the floor plate and lower two thirds of the developing spinal cord, the netrins may attract commissural axons.

As the worm's axons turn. These two new axon guidance assays provided the field with some of their best leads in years. One was found in the laboratories of Thomas Jessell and Jane Dodd, both at Columbia University. Their groups were exploring the role of the floor plate in neural development. By defining the midline of the developing nervous system, this slim piece of embryonic tissue helps establish the left and right sides of the spinal cord and the brain. And as far as axon guidance goes, the midline is "where important decisions are made," says Mark Fishman of Massachusetts General Hospital. For instance, while some axons avoid the midline, others start on one side of the embryo, saunter across the midline, then make a sharp turn for the brain.

In the late 1980s, Columbia researchers Jessell, Dodd, Tessier-Lavigne, then a postdoc, and Marysia Placzek were investigating a population of rat spinal nerve cells called commissural neurons. These neurons lie in the dorsal (upper) part of the embryonic spinal cord, but their axons follow a circumferential path that sends them down toward the floor plate on the ventral side of the spinal cord, where they cross the midline and head towards the brain. Using the Lumsden and Davies assay, the labs quickly showed that the floor plate was secreting something that gained the attention of the nomadic axons.

The next step was to isolate that something—a task that took 6 years. Because of the floor plate's lilliputian size, researchers simply couldn't get enough of the tissue to isolate the chemoattractant. As a result, researchers began screening cultured cell lines and samples of embryonic and adult tissues, looking for something more substantial that reproduced the floor plate's effect on commissural axons. Tessier-Lavigne, who had meanwhile moved to UCSF, and his lab eventually hit on brain tissue of young chicks, and from a solution made of 25,000 pulverized chick brains they purified two novel proteins, netrin-1 and netrin-2.

After using the amino acid structure of these proteins to find and clone their genes, they confirmed that the netrins are chemoattractants by genetically engineering a non-neuronal cell line to secrete the proteins. Commissural axons, they found, turn towards the altered cells just as they do to normal floor plate tissue. The growth cones of these axons more than likely sport receptors that bind to netrin molecules and signal axons to grow towards concentrations of the proteins.

It was around that time that worms wriggled into the picture, says Tessier-Lavigne. While his collaboration had strong proof that netrins could attract axons in petri dishes, there was no direct evidence that they played any such role in an actual organism. But when postdocs Timothy Kennedy and Tito Serafini ran the sequences of the cloned netrin genes through a computer database, it flagged *unc*-6, a gene found in the nematode *Caenorhabditis elegans*. Despite the vast differences between vertebrates and invertebrates, which split more than 600 hundred million years ago, the protein made by *unc*-6 shares more than 50% of its amino acid sequence with the netrins. "It was exhilarating," says Tessier-Lavigne.

The lab's excitement stemmed from the fact that *unc*-6 had already been implicated in axon guidance in C. *elegans*. In the late 1980s, a team including Joseph Culotti of

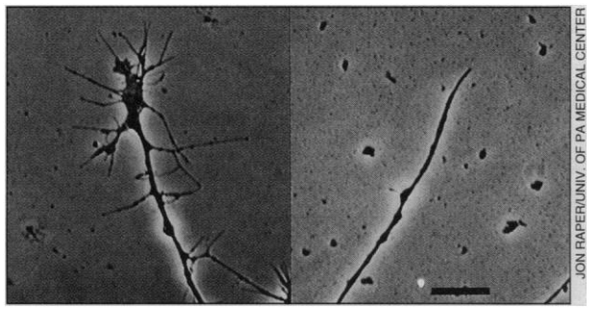
Toronto's Mount Sinai Hospital Research Institute, Ed Hedgecock of Johns Hopkins University, and David Hall of Albert Einstein College of Medicine first described in detail a nematode mutant discovered years earlier. In this mutant, they found that axons that normally travel circumferentially around the worm, from dorsal to ventral or ventral to dorsal, get misrouted. By 1992, they had cloned *unc*-6, the gene causing the mutation (unc stands for uncoordinated). While this work strongly suggested that Unc-6 is a guidance molecule, it did not prove it.

But the discovery of the structural similarity between the netrins and Unc-6 makes it likely that the protein is indeed a chemoattractant molecule for some axons in the nematode. And conversely, the axon guidance defects in the *unc*-6 mutants provide compelling evidence that the netrins will be found to guide commissural axons circumferentially during normal development of vertebrates. Further support for this hypothesis came from Tessier-Lavigne's group, when they showed that the netrins are produced in the right locations in the spinal cord to guide the axons around the cord to the floor plate. Full proof won't come, however, until researchers "knock out" the netrin genes and show that their loss does disturb axonal migration.

The netrins and *unc*-6 aren't the only hot story in axon guidance that brings together invertebrates and higher organisms. Last year, in the 22 October issue of *Cell*, Raper's lab, now at the University of Pennsylvania School of Medicine, described a new protein, purified from chick brain, that they called collapsin because it causes growth cones to collapse. Like the netrins, collapsin was another intriguing protein that affected axons in the test tube, but interest in it went to another level when Raper's lab cloned the gene and ran the sequence through the database.

The result showed that the DNA sequence of the *collapsin* gene bore a strong resemblance to the gene for a grasshopper protein, then known as Fas IV, that had been discovered in Goodman's lab in 1992. That year, his lab, in collaboration with David Bentley's at UC Berkeley, also showed that Fas IV played an in vivo role in grasshopper growth cone guidance by blocking the protein with an antibody. Goodman's lab has since shown that Fas IV's gene is actually part of a gene family they call semaphorins that is found in grasshoppers, fruit flies, and even mammals. Because Fas IV, now called Sema I, is related to collapsin, these results make researchers confident that collapsin plays a physiological role in axon guidance, even though they have yet to confirm it.

Still, there are many questions about how collapsin fits into the puzzle of axon guidance. Raper's lab, for instance, had thought it was isolating a membrane-bound protein, but collapsin is secreted by the cell. Perhaps collapsin is a repulsive cue that, rather than being anchored on a cell surface, is diffused freely through the developing embryo. A couple of years ago that would have seemed an odd concept, but last year in the 2 July issue of *Science*, Adrian Pini of the Medical Research Council Mammalian Development Unit in London reported that the mammalian nervous system secretes diffusible chemorepulsive cues in addition to chemoattractive ones.



JON RAPER/UNIV. OF PA MEDICAL CENTER

Nervous breakdown. The healthy growth cone *(left)* of an axon will shrivel up *(right)* when exposed to the protein collapsin.

Is collapsin one of those negative cues? That's unclear, because the protein appears to bind quickly to the extracellular matrix and the surfaces of cells, which would prevent it from diffusing very far. Another prospect is that collapsin provides a short-range inhibitory cue that prevents the axon from splitting into multiple fibers until it nears its target, a branching phenomenon that O'Leary's lab has taken the lead in exploring.

Double agents. One of the most important themes emerging from the recent flurry of papers about axon guidance may be that the molecules that steer growth cones actually lead double lives, beckoning some axons while repelling others. Take connectin, a fruit fly protein discovered by Goodman's lab in 1992. Goodman's group and others had suggested that this cell adhesion molecule provides attractive cues to some types of motorneurons. But their latest results, published in the 23 September issue of *Neuron*, indicate that connectin wards off or stalls growth cones of other motorneurons.

Connectin's two-faced personality may be shared by many other guidance cues. Studies indicate that while *unc*-6 may attract some neurons toward the ventral region of the worm, where it is believed to be most concentrated, it may rebuff other axons, causing them to move towards the dorsal side. Inspired by those results, Tessier-Lavigne's lab is vigorously investigating whether the netrins complement their ability to attract some axons with the ability to repel others.

Developmental neurobiologists now suggest that how an extending axon reacts to any specific guidance molecule depends on the kinds of receptors that protrude from its growth cone. One type of netrin receptor might interpret the protein as an attractant; another would send a signal into the axon telling it to retreat. "That way you can have different axons responding differently to the same target. It's a really nice way to build things," says Goodman, pointing to the midline as one area where the same tissue seems to provide disparate directions to different axons.

Though the hypothesis that most guidance molecules lead double lives is appealing, it is far from proven, largely because of a dearth of knowledge about the proteins growth cones sport. "Identifying the receptors on the growth cone will be a major focus of the future. That story is still wide open," says Roger Keynes, a developmental neurobiologist at the University of Cambridge, U.K. One of the first chapters in that story may come again from the nematode: Researchers have identified a gene called *unc*-5 that appears to make a growth cone receptor that recognizes Unc-6 and tells the extending axon to avoid it, says Culotti.

Much like earlier neurobiologists, who first had to identify a slew of neurotransmitters before they could start to understand how the brain conveyed messages, developmental neurobiologists are just now taking the first real steps towards understanding the journey of an axon. Many guidance molecules have yet to be discovered, says Keynes, and "every single candidate has to be fit into a big picture." Indeed, considering the importance of its task, the wiring of the nervous system, most expect that nature's axon guidance machinery will have built-in redundancy that will make the function of any single part difficult to understand."A huge amount of work remains to be done," says Herman Stellar, who studies axon guidance at the Massachusetts Institute of Technology.

Still, the insights of the last 2 years, especially the apparent conservation of axon guidance cues between invertebrates and vertebrates, has stimulated a new optimism among those who study how the nervous system wires itself. "The field will now advance rapidly because we can go back and forth [between research organisms]," says Goodman. From the worm to the rat, from the fruit fly to the chicken, it appears that some of Ramón y Cajal's scents are being sniffed out.

–John Travis

Additional Reading

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H. Baier and F. Bonhoeffer, "Attractive axon guidance molecules," *Science* **265**, 1541 (1994).

C. Goodman, "The likeness of being: Phylogenetically conserved molecular mechanisms of growth cone guidance," *Cell* **78**, 353 (1994).