NEUROBIOLOGY

Getting a Handle on the Molecules That Guide Axons

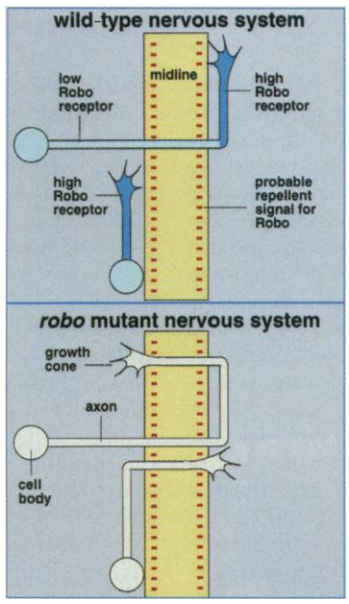
Some nerve cells in a growing embryo act like people with an all-consuming crush. They zero in on the object of their attraction, the midline of the embryo, with seemingly single-minded focus. But, like many lovers, the neurons are fickle. Once their axons, the long projections they send out to contact the midline, have achieved intimacy with it, many ignore—or even spurn—the object of their obsession. Instead of remaining at the midline, the axons continue to grow, searching for their ultimate destiny elsewhere in the nervous system, where they will make specific connections with other cells.

This switch in affections is critical because it allows axons to cross the midline so that the two sides of the nervous system can talk to each other, but it has mystified scientists. Now, work from several labs is revealing some of the molecular logic that enables axons to change their conduct so abruptly.

Developmental biologists have discovered in the last several years how growing axons find their way to the midline in the first place: They are drawn in by attractive molecules, among them the proteins called netrins, released by midline cells. But realizing this only deepened the mystery of how the axons manage to continue on their journey. For example, certain kinds of axons grow across the midline and then turn sharply to run along the length of the body on the other side—and they never cross back. "These cells never think about the midline again," says neurobiologist Tom Kidd, a postdoctoral fellow in Corey Goodman's laboratory at the University of California, Berkeley. "They forever ignore the signal that most strongly attracted them early in life."

The new results, which appear in five papers published this month and last in *Cell*, *Neuron*, and *Science*, are now revealing how axons complete their journeys through and beyond the midline. While many pieces of the axonal guidance puzzle remain to be found, together the papers show that a dynamic interplay of both attractive and repellent signals between the midline and the nerve cells themselves directs axon movements. For example, axons carry a surface protein called Robo that can prevent them from crossing the midline, apparently because it is the receptor for an as-yet-unidentified repellent molecule. But the midline itself makes another protein, known as Comm, which reduces Robo's concentration, allowing the axons to traverse the midline. Afterward, Robo's concentration shoots up, preventing the axons from retracing their steps.

The midline also makes axons lose their ability to respond to the alluring signals that brought them there. Indeed, there are even indications that axons can completely change the nature of their responses—being repulsed instead of attracted by signals such as netrin-1. "These papers have shown very nicely that even a simple decision—whether or not to cross the midline—is actually very complex," says developmental neurobiologist Lynn Landmesser of Case Western Reserve University in Cleveland. "The axon has to integrate multiple kinds of guidance cues, some positive and some negative."

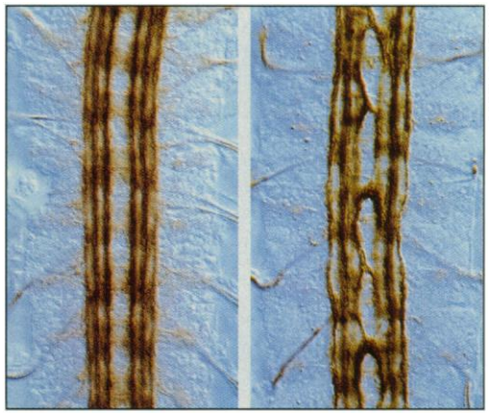


COREY GOODMAN

Crossing guard. Neurons with low Robo concentrations can cross the midline of the embryo, but an increase in the protein levels prevents them from crossing back. In contrast, mutants lacking the protein cross freely, even when they wouldn't otherwise.

Researchers hope that what they are learning about the molecules that guide axon behavior at the midline will eventually lead to a better understanding of how nerve cells know where to go as they set up the entire nervous system, including the brain and neuronal connections in the periphery. It could also lead to ways to tackle important medical problems, such as why damaged spinal cords don't regenerate. "If we knew more about what makes axons grow or inhibits them, we could understand why axons in the nervous system of a fully developed human being won't grow," says developmental neurobiologist Tom Jessell, of Columbia University's College of Physicians and Surgeons in Manhattan.

Researchers have focused on the midline because axonal behavior there is so easy to follow. "Axons behave in very dramatic and very stereotyped ways at the midline. It's easy to see when something is wrong," says Marc Tessier-Lavigne of the University of California, San Francisco. Indeed, in the early 1990s, Guy Tear and Mark Seeger, who were both postdoctoral fellows in Goodman's lab at the time, took advantage of that stereotypical behavior to identify the genes that feature in the current work.



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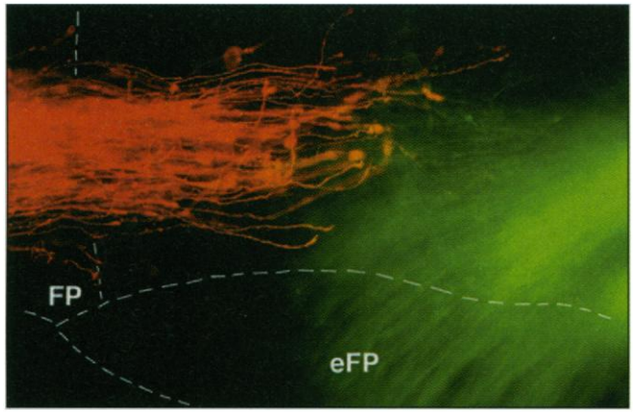
Losing their way. Although a few axons (brown stain) don't normally cross the fruit fly midline *(left)*, in *robo* mutants (right) they weave back and forth.

They screened tens of thousands of mutant fruit fly embryos, looking for those in which either too few or too many axons crossed the midline. Two superstar mutants emerged from those experiments: *roundabout* (*robo*), so named because the axons in animals meander back and forth across the midline, flagrantly disregarding the barrier that normally separates the animal into its two halves, and *commissureless* (*comm*), which displays the exact opposite behavior. Instead of crossing the midline and creating bridges, or commissures, between the halves, axons in *comm* mutants run straight up and down on both sides.

By 1996, Seeger and Goodman's teams had cloned the *comm* gene and shown that its protein product resides on the surface of midline cells. They had also found that while Comm's presence there is normally essential for axons to cross the midline, it is not required when the *robo* gene is defective as well. In that case, axons in fruit fly embryos weave back and forth just as they do when only *robo* is faulty. That observation suggested that Comm and Robo somehow collaborate to keep axons on the right track. But how the two proteins might cooperate wasn't clear until now.

In work described in the 23 January issue of *Cell*, Kidd, Tear (who is now at Imperial College in London), Goodman, and their colleagues have cloned the fruit fly's *robo* gene. Sequence analysis suggests that the protein encoded by the gene is a receptor, translating signals from the environment into decisions about how the axon should move. The researchers have not yet identified that signal, however.

To get a better indication of what the protein does, Goodman and Tear's groups stained axons with antibodies that either detect Robo or bind to proteins specific to subpopulations of axons that have distinctive trajectories. That procedure enabled them to see that the rare axons that normally never cross the midline carry large amounts of Robo on their surfaces. Those that do cross display low levels of the receptor until after they have reached the other side. At that point, the axons dramatically turn up Robo production. These results, along with the observation that axons freely cross and recross the midline in mutants lacking Robo, suggest that the protein detects a chemical that deflects the axon tip from the midline.



SHIRASAKI *ET AL.*

Turnoff. Axons that have not crossed the midline (FP) of the rat-brain strip (green) turn toward a second midline tissue (eFP), while those that have crossed the midline (red) ignore the extra piece.

Comm enters the picture because it apparently turns down Robo's concentration so that axons can cross the midline, the researchers report in the January issue of *Neuron*. When they genetically engineered embryos to produce Comm in large quantities, they found reduced levels of Robo on axons, which crossed the midline unrestrained, just as they do in embryos missing the *robo* gene itself. "This is very exciting, because it shows that cells at the midline can influence how an axon can change its whole personality as it crosses," says neurobiologist David Van Vactor of Harvard University. Those axons destined to remain on one side of the midline presumably have so much Robo from the outset that Comm can't overcome the large quantities present.

The researchers are now trying to figure out exactly how Comm works. They don't know, for example, whether it interacts with other factors that help turn down the amount of Robo on the axon surface. Also, the predicted chemical, presumably made at the midline, to which Robo responds is still at large.

But whatever its identity, other work indicates that the Robo-Comm axonal guidance system is widespread in the animal kingdom, suggesting that evolution developed this system for allowing axons to cross the midline and then exploited it over and over again. In an independent investigation, also described in the 23 January *Cell*, Jennifer Zallen, a graduate student in Cori Bargmann's lab at the University of California, San Francisco, studied nematodes—tiny roundworms—to find mutant strains in which axons stray from their normal trajectories during development. One such strain also turned out to resemble *robo* fruit fly mutants, in that some axons zigzagged back and forth across the midline. When Bargmann and her colleagues isolated and sequenced the gene at fault, which they call *Sax-3* because it normally helps keep sensory axons on track, they found that the sequence of the protein it encodes closely resembles that of the fruit fly's Robo.

"It's very encouraging that work from two very different kinds of mutant screens in two different organisms came up with the same key gene, especially given that flies and worms have nervous systems that superficially look quite different," says Goodman. His team and graduate student Katja Brose in Tessier-Lavigne's lab have now also cloned the corresponding genes in the rat and human, which encode proteins that look very much like Robo. "It's not a big leap of faith to imagine that the homologous genes will function in the same way in mammals," Goodman adds.

How neurons can move through the midline at all, given the continued presence of the attractive chemicals that drew them there in the first place, has also been a puzzle. A possible answer comes from Fujio Murakami's group at Osaka University in Japan: The midline itself somehow causes rat axons to become unresponsive to the enticing properties of these attractants as the nerves cross over.

In work described in the 2 January issue of *Science*, postdoc Ryuichi Shirasaki placed strips of brain with the midline removed near a second piece of brain containing the midline. He found that fluorescently labeled axons from the first strips grew toward the second, indicating they were being attracted by a chemical there. But when he left the midline in the first piece, the axons that crossed it completely ignored the second piece of midline tissue, even though they were close enough to sense the chemicals emanating from it. One of the chemicals that the axons no longer sensed seems to be netrin-1, because when Shirasaki repeated the experiment using netrin-1–producing cells in place of the second midline, the axons ignored those cells just as they had ignored the midline tissue itself.

Neurons may do more than just lose their responsiveness to netrin-1 when they cross the midline; they may even become repelled by it. When Mu-ming Poo's and Christine Holt's groups at the University of California, San Diego, in collaboration with Tessier-Lavigne, subjected isolated nerve cells in culture to a gradient of netrin-1, they found that the axons normally migrate toward higher concentrations of the signaling molecule. But when the researchers inhibited an enzyme called cAMP-dependent protein kinase A, which plays an important role in the cell's internal signaling pathways, the axons changed their behavior, veering away from netrin-1 instead of moving toward it. At this point, the researchers don't know whether the same thing happens in the developing nervous system, but the result, which appeared in the December issue of *Neuron*, shows how flexible axons' responses to guiding molecules can be. "The same axon can find a cue attractive or repulsive, or it can ignore it," Tessier-Lavigne says.

Researchers are still a long way from having the complete list of the molecular interactions that choreograph developing axons. But they say that the progress they've made so far has brought them to the edge of the next big frontier: sorting out how individual nerve cells synthesize the many signals they receive. This will require delving into the inner workings of the axon tip to understand how receptors such as Robo influence the filaments of the neuron's internal skeleton (cytoskeleton) to cause an axon to turn. "Once we have a handle on the signaling machinery that links receptors to the signaling machinery, we will be able to understand where and how the effects of these signals are integrated," Tessier-Lavigne says. In the meantime, unlike most fickle humans, nerve cells are revealing at least some good explanations for their quirky behavior.

–Evelyn Strauss

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