DEVELOPMENTAL NEUROBIOLOGY

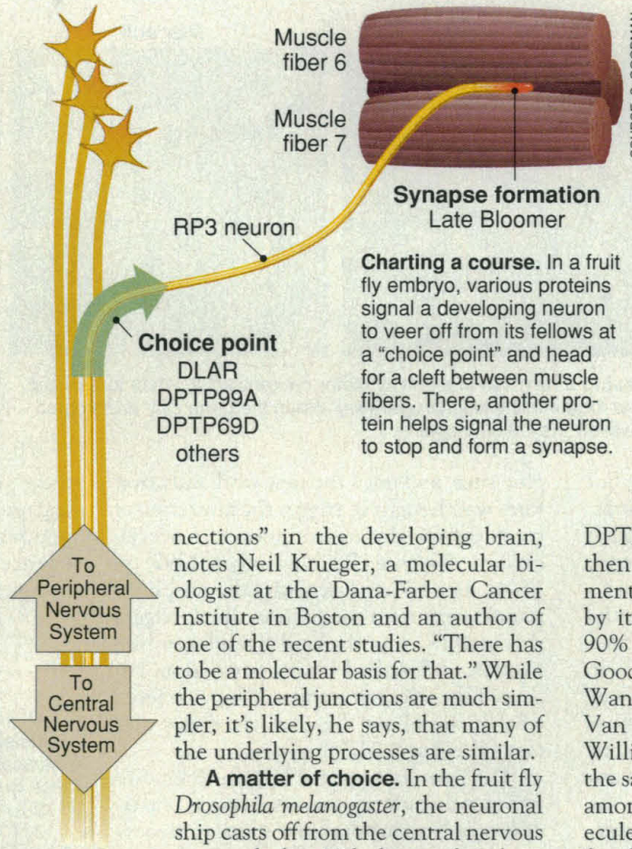
# New Neurons Use "Lookouts" To Navigate Nervous System

Like a ship journeying toward a strange and distant harbor, a growing motor neuron heading into the peripheral nervous system faces a navigational challenge. In these uncharted waters, the neuron's growing extension, or axon, must find the one channel leading to its ultimate port: the muscle group that it is destined to control. Once it is on the right heading, it has to cut its engines at the proper time or risk overshooting the dock. And when finally tied to the wharf, it needs to mobilize dock workers on the muscle cells to unload its precious cargo of neurotransmitters.

Scientists, in their attempts to discover how a neuron does all this, have been a little at sea themselves. But in a series of recent studies of developing fruit fly embryos, researchers at several labs have spotted some key members of the neuron's molecular crew. They have identified a handful of lookouts on deck—proteins that straddle the cell membrane—that help guide the axon on its journey, signaling its leading edge, known as the growth cone, to swerve away from the main nerve channel and toward its target muscle. Then, according to a report on page 1867 in this issue of *Science*, a newly discovered protein called Late Bloomer helps to signal "All stop" once the growth cone has docked in the muscle fibers. And studies of the rat have also identified several proteins that serve as the dock workers, setting in motion the molecular gangplanks and cranes that off-load the neurochemical cargo.

Taken together, these findings "start to paint a nice picture of the kinds of molecules motor neurons use to exit the central nervous system, then stop at the right targets and make the proper synaptic connections," says Nipam Patel, a developmental biologist at the University of Chicago. With the identities of some of the lookouts pinned down, scientists can now use them to help fill in other parts of the picture. In particular, they would like to find the still-mysterious navigational signals the lookouts pick up, as well as the messages that they relay in turn to the cell's interior.

Eventually, researchers say, the lookouts may help neuroscientists chart a far more intricate journey. These molecules and a suite of others that have been discovered in the past few years, such as Eph receptor kinases, netrins, and semaphorins, are beginning to yield clues to how the nervous system comes together not just in the periphery, but in the brain itself. "There are trillions of neurons making quadrillions of neural connections" in the developing brain, notes Neil Krueger, a molecular biologist at the Dana-Farber Cancer Institute in Boston and an author of one of the recent studies. "There has to be a molecular basis for that." While the peripheral junctions are much simpler, it's likely, he says, that many of the underlying processes are similar.




SOURCE: C. GOODMAN

**Charting a course.** In a fruit fly embryo, various proteins signal a developing neuron to veer off from its fellows at a "choice point" and head for a cleft between muscle fibers. There, another protein helps signal the neuron to stop and form a synapse.

**A matter of choice.** In the fruit fly *Drosophila melanogaster*, the neuronal ship casts off from the central nervous system around the 10th hour of embryo growth. Bundles of axons leave the ventral nerve cord—equivalent to the vertebrate spinal cord—and set sail for muscles in each of the fly's various body segments. Each axon in a bundle makes a crucial course correction as it approaches the target muscles. In this area, called the "choice point," the axon must peel off from the bundle and find its own way into the cleft between muscle fibers.

Hoping to identify the molecules involved in this choice, researchers in the laboratories of neuroscientists Kai Zinn at the California Institute of Technology (Caltech), Haruo Saito at Dana-Farber, and Corey Goodman at the University of California, Berkeley, have been looking for genes that disrupt the process when mutated. The scientists had three likely candidates: genes encoding three *Drosophila* proteins, DLAR and DPTP69D—identified in Saito's lab in 1989—and DPTP99A, found by Zinn and others in 1991. These molecules are well-positioned to act as lookouts, relaying external signals to the cell's interior. All three belong to a membrane-spanning family of proteins known as receptor tyrosine phosphatases, which can be activated by extracellular signals and then turn on intracellular messenger molecules by removing their phosphate groups. What's more, antibody staining of the *Drosophila* proteins showed that all three are expressed exclusively in the fly embryos' nervous systems.

In the 23 February issue of *Cell*, Caltech neuroscientist Chand Desai, Zinn, and molecular geneticists Lawrence Goldstein and Joseph Gindhart of the University of California, San Diego, report that mutations in the gene encoding DPTP69D produced embryos whose motor neurons got lost in about one fifth of all body segments. These axons did form synapses, but in muscles far downstream of their proper destinations, as if the growth cones never received the signal to turn aside. Instead they sailed blithely onward, bypassing their targets.

The researchers found they could make the problem even worse by deleting sections of the gene encoding DPTP99A at the same time. The neurons then went astray in nearly all body segments. Deleting the gene encoding DLAR by itself causes similar trouble in 80% to 90% of the segments, as Kreuger, Saito, Goodman, Berkeley neurobiologist Hong Wan, and Harvard neurobiologist David Van Vactor and developmental biologist William Gelbart report in another paper in the same issue of *Cell*. Says Zinn: "These are among the first examples of surface molecules that, when disrupted, give you defined errors in axon guidance."

Because similar misguided journeys are seen in mutant embryos that produce too much fasciclin II—a protein that helps axons stick together in bundles or "fascicles"—Zinn suggests that the role of these phosphatase molecules is to pass on signals that prompt neurons to unstick themselves and bend away from the bundles. This scenario makes even more sense in light of DLAR's similarity to a human cell-surface phosphatase called LAR, which is known to interact with molecules that regulate the skeletons of epithelial cells lining blood vessels, signaling the cells to change shape and migrate when necessary. "Whatever is being dephosphorylated by DLAR is giving the [neuronal] cytoskeleton directions to

start moving here or stop moving there," concludes Krueger. He and other researchers now intend to use DLAR as a probe to reveal that unknown "whatever" molecule and thus move up the neuronal ship's chain of command.

**Local waters.** Once the neuronal vessel has left the main channel, it depends on a multitude of attractive and repulsive molecular signals from its surroundings—including, for example, the muscle membrane attractant fasciclin III and the muscle-secreted chemorepellent semaphorin II—to locate its target muscle. The neuron must then slow down and nestle up to this wharf in order to form a synapse.

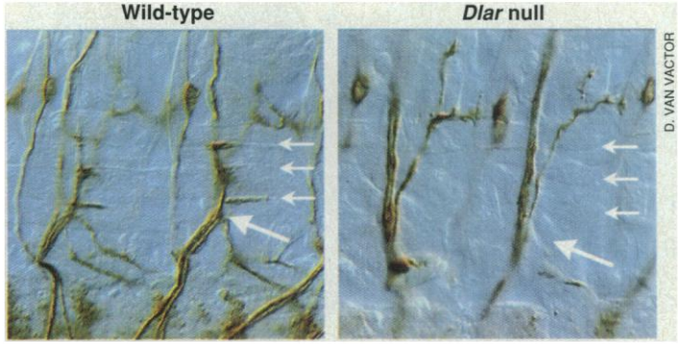
That task requires a local pilot, and Goodman's group at Berkeley seems to have found one. Casey Kopczynski, a neuroscientist in Goodman's lab, was searching for *Drosophila* cell-surface proteins that might help the growth cone slow down and transform into the treelike "terminal arbor" that makes up the neuronal side of the synapse. When he found a novel motor neuron protein that, when mutated, had almost the opposite effect, he knew his search had succeeded. On page 1867, he and his colleagues report that growth cones in these mutants found their way to the correct target muscles, but didn't retract their crawling extensions, called filopodia, or form arbors until much later in the developmental process—hence the name Late Bloomer (LBL).

The protein is a member of the "tetraspanin" family, a group of membrane-spanning proteins that, in mammals, help to slow down cells in several ways. They stimulate cells to stick to their surroundings and also inhibit changes in cell skeletons and shapes that would otherwise allow cancer cells, for example, to creep about the body. Decreasing cell mobility and increasing cell adhesion, notes Goodman, "are exactly the sorts of functions you'd like to see" in a protein that helps to develop a synapse.

As with the axon-guiding phosphatases, LBL's biochemical partners—the outside signals and interior messengers—remain unknown. But it's unlikely that finding these partners will complete the story. Because synapses do ultimately form in embryos lacking LBL, it appears that the protein may only help this process along as part of a larger complex of molecular lookouts, says Goodman. "What we're hoping," he says, "is that in the future, through either biochemistry or genetics, we can discover what the other elements of this complex are"—and whether comparable mechanisms promote synapse formation in the brain.

**Ship to shore.** Once the vessel does come to a halt, the adjacent muscle fiber has to mobilize the equipment to receive the neuron's cargo: the neurotransmitters released by the branches of the terminal arbor. According to a paper scheduled to appear in the May issue of *Development*, stevedores on the muscle-cell dock and crew members on the axonal deck may take part in setting up this off-loading equipment, which takes the form of cell-surface receptors.

The neuron's contribution to this work gang had already been identified: agrin, a protein secreted in different forms by neurons and muscle cells. The neuronal agrin features extra amino acids spliced into specific sites, and until the new work only that form was thought to trigger the clustering of receptors for the neurotransmitter acetylcholine (*Science*, 20 November 1992, p. 1304). But no one knew why the unspliced muscle version of the protein didn't trigger this activity, says Richard Scheller, a biochemist at Stanford University and an author of the upcoming paper: "We wondered what it was about these spliced forms that made them special."




**Missing a turn.** Normal fruit fly motor neurons know when to turn and head for a muscle junction *(arrows)*. When the gene *Dlar* is disrupted, however, they keep on going.

The neuronal agrin is known to bind to two muscle surface proteins, heparin and α-dystroglycan, that may in turn trigger receptor clustering. So Scheller and colleagues James Campanelli and Gregory Gayer designed experiments to compare the abilities of both muscle and nerve agrin to bind to these two proteins. A spliced agrin did bind more tightly to heparin than did other forms. But then the researchers got a surprise: Agrin with no spliced inserts—the muscle-secreted form—bound to the other protein, α-dystroglycan, far more strongly than did neuronal agrin.

"The interpretation of that result is a little bit up in the air," Scheller says. It may mean, however, that muscle- and neuron-secreted forms of agrin must somehow work together to activate receptor clustering. He speculates that unspliced agrin may be the main substance that binds to α-dystroglycan, which may in turn stimulate the muscle-cell cytoskeleton to drag acetylcholine receptors together into clusters. But he thinks the spliced forms may have to bind to another unknown receptor at the same time, possibly with help from heparin, to activate clustering fully. Yet another possibility, says University of Colorado neuroscientist Bruce Wallace, is that "α-dystroglycan and heparin are not directly involved in clustering. If these molecules serve any role at all, it may be a secondary one, perhaps helping to concentrate agrin in the vicinity of the real receptor." Whatever the case, say Scheller and other researchers, such "coordinate regulation"—involving multiple, distinct signals and surface molecules—is likely to be a key aspect of nervous system development in many organisms, and perhaps also in the brain.

How far have the recent studies carried neurobiologists toward that ultimate destination? "Clearly," says Chicago's Patel, "the human brain is going to have orders of magnitude greater complexity" than the neuromuscular synapse. "But these model systems are providing invaluable insights into how something like that works. And given all the conservation we're seeing in biochemical pathways from *Drosophila* to humans, there's almost no doubt these kinds of genes and proteins will be important in understanding any nervous system." Neuroscience's ship, in other words, is gradually coming in.

–Wade Roush


Additional Reading

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