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## New Class of Molecular Cues Guides Nervous System Wiring

In the developing nervous system, a group of molecular road signs directs growing nerve cells toward their correct destination. Now, collaborating groups of Howard Hughes Medical Institute (HHMI) investigators have discovered a new type of road sign that depending upon the condition either repels growing neurons or triggers neurons to sprout new connections.

As reported in three articles in the March 19, 1999, issue of the journal *Cell*, the seemingly contradictory "Stop-and-Go" behavior of the protein Slit opens an intriguing new chapter in understanding how the nervous system is wired, say the scientists. If the researchers can understand the machinery controlling such wiring decisions, their insights may lead to new approaches to regrow or repair damaged or severed spinal cord nerves.

The *Cell* articles describe how the two HHMI laboratories, headed by long-time friends and scientific collaborators Corey Goodman and Marc Tessier-Lavigne worked together closely to study the Slit protein in two very different organisms: fruit flies and mammals.

A repellent signal for axons, Slit (in red), and its receptor, Robo (in green), help determine whether an axon crosses the center or "midline" of the fruit fly nervous system.

In a first set of studies, Goodman, an HHMI investigator at the University of California at Berkeley, and HHMI associate Tom Kidd and colleague Kim Bland used genetic mutant screening studies of the fruit fly *Drosophila* to pinpoint the Slit protein as a key repellent molecule in developing fly embryos. The group had previously shown that the roundabout, or Robo, protein is a repulsive receptor on the surface of growing axons. They also demonstrated that Robo controlled whether axons crossed or recrossed the center, or "midline," of the fruit fly nervous system to properly connect the brain's two halves by responding to an unknown midline repellent signal.

In the current study, Goodman and his colleagues were searching for that midline repellent signal and found that the Slit protein prevented neurons that had crossed the midline from crossing back over. Once axons cross the midline, they turn up their levels of Slit receptor (Robo) and this prevents them from crossing again, says Goodman. Thanks to the Slit protein, which Goodman and his colleagues in the first of the three *Cell* articles call the

"midline repellent," the developing brain avoids a tangle of catastrophic miswiring.

These investigators then collaborated with HHMI investigator Marc Tessier-Lavigne and colleagues at the University of California San Francisco to explore Slit's function in mammals, extending the work in *Drosophila*. In the second *Cell* article, predoctoral fellow Katja Brose and others in Tessier-Lavigne's and Goodman's laboratory reported finding mammalian versions of Slit, which also act as repellents for growing mammalian neurons.

But that was not the end of the Slit story. In another set of studies, Kuan Hong Wang, an HHMI predoctoral fellow in Tessier-Lavigne's laboratory, had been working independently at a lab bench next to Brose's for several years on what they thought was a separate project. While Brose was isolating the mammalian versions of Slit in collaboration with the Goodman laboratory, Wang was attempting to isolate an unknown protein that induced branching of neuronal axons, the cable-like structures that neurons grow to establish contacts with other neurons.

When Wang finally isolated the branch-inducing protein and determined its structure, he and Brose realized that their two molecules were basically identical. "At that point, we fell off our chairs," said Tessier-Lavigne. "It's one of those amazing moments in the laboratory when you think you're working on two different things, but in fact you're working on two different faces of the same thing."

Both Goodman and Tessier-Lavigne view the serendipitous convergence of these two lines of study, reported in the third *Cell* article, as opening a promising new pathway to understanding the intricacies of how the brain and nervous system is wired.

"We know that neurons grow through a series of choice points, like driving a car to a destination," said Goodman. "You don't just dead-reckon straight to your destination. You turn onto one road, then turn onto another, make turns at a series of intersections, and finally arrive at your destination."

Neurons, like automobile drivers, depend on road signs, adds Tessier-Lavigne, and for neurons the Slit protein is clearly an important neural road sign. "You can think of guidance molecules such the Slit protein as a sign or an arrow pointing in a particular direction," he said. "A neuron can respond to that arrow in one of three ways: be attracted into going in the direction of the arrow, be repelled into going in the other direction, or just ignore it altogether."

The growing neurons likely decide on their response to these guidance proteins through sensors or receptors on their tips, called growth cones. These receptors allow the growing neuron to "read" the sign posts in the developing brain. In the case of Slit, the researchers already have an inroad into understanding how the signal is read by the growth cone because they had previously identified its repulsive receptor, Robo.

The concept of multiple responses to the same guidance molecule has already been confirmed in other axon-guiding molecules, including families known as netrins, ephrins and semaphorins, say Goodman and Tessier-Lavigne. The Slit proteins, however, constitute a new family of such proteins, offering yet another pathway for exploring the intricacies of neuronal wiring.

The HHMI scientists will devote future research efforts to exploring the control machinery of the Robo receptor and Slit, attempting to understand in detail how Slit acts as a repellent in some cases and an axon-branching promoter in others.

Their hope is that such knowledge will enable them to use one set of chemicals to persuade severed neurons, as in damaged spinal cords, to produce new growth cones capable of reacting to Slit as a growth promoter. If they succeed, such neurons may be able to form new functional connections, and thus, restore lost nervous system function.