NEUROBIOLOGY

The Come-Hither, Don't-Touch-Me Proteins

Two newly discovered receptors help determine how far axons in developing fruit flies travel after they cross the line between the fly's left and right sides

Finding a path in life involves a vast array of small choices, each of which can lead in a new direction. Neurons in the developing nervous system face similar choices as they send out their axons—the long extensions that reach from the cell body and communicate with other neurons. At the individual level, each axon's growth looks spontaneous, yet, in complex organisms, trillions of neurons all find their way in a highly controlled pattern. There are occasional mistakes, but the system usually ends up wired correctly.

Over the years, researchers have identified several pieces in the puzzle of how neurons establish their specific connections, and this week in *Cell* and *Neuron*, two teams working with the fruit fly help clarify the picture. The work solves several old mysteries about how insect axons find their proper targets.

In the developing fruit fly, many neurons on one side of the midline—the dividing line between left and right sides of the fly—send their axons across the center to the other side. At first, the midline seems to be a keen object of attraction for such axons, but once they reach the midline, they don't stay. In fact, the midline becomes repulsive, and the axons keep their distance, never crossing it again.

A few years ago, scientists discovered a suite of proteins that control this fickle pursue-and-reject pattern. Proteins known as netrins attract the axons toward the midline, which also produces a repellent protein called Slit. The axons don't initially sense the repellent, but once they cross the midline they begin to express a receptor protein for Slit called Roundabout (Robo). Slit repels Robo, and so the traversing axons continue on their way and never look back. This elegant system allows the two sides of the nervous system to communicate in the adult animal.

Now, it seems the Robo-Slit dance involves a few more partners. In a pair of papers in the December issue of *Neuron*, two independent teams—led by Barry Dickson of the Research Institute of Molecular Pathology in Vienna and Corey Goodman of the University of California, Berkeley—report that flies have two proteins very similar to Robo, called Robo2 and Robo3. In two more papers in this week's issue of *Cell*, both teams describe how the Robo proteins on an axon's cell membrane not only keep the axon from recrossing the midline but also help determine the particular path an axon takes.

Scientists had suspected that Robo might have relatives. In fruit fly embryos that lack the repulsive Slit, axons crowd together at the midline. But those missing Robo behave differently: Their axons cross the midline, then turn back and cross it again. That difference in behavior suggested that another receptor also responds to Slit's repulsive cue, but the signal is too weak on its own to keep axons on the correct path. Both groups searched the newly completed fruit fly genome for proteins that resembled Robo, and both found the two relatives, Robo2 and Robo3.

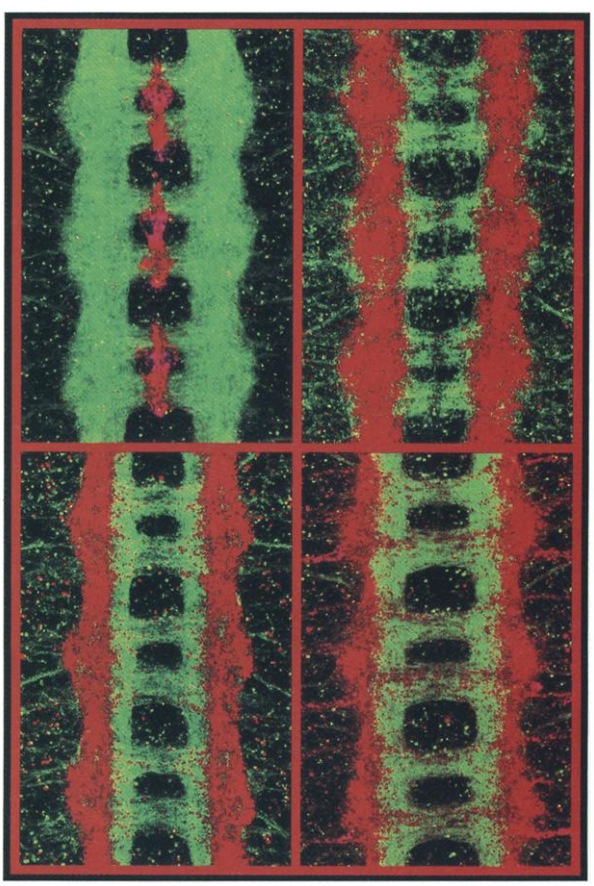
The researchers wondered if the combination of Robo receptors on an axon might help determine how far it travels from the center line. That's exactly what they found: Axons that express only Robo suddenly turn and travel on a path parallel to the center line, staying in a zone closest to the middle, where the concentrations of Slit are still relatively high. Those that express both Robo and Robo3 travel slightly farther from the midline, to an area of lower Slit concentration. And those that express all three receptors travel the farthest.

Genetic experiments support the model. When the teams created fly embryos that lacked Robo2, the axons most distant from the midline did not travel as far; instead, they joined an intermediate path. When the researchers interrupted the production of Robo3, the intermediate path disappeared. The innermost pathway had more axons than usual, while the outermost pathway seemed normal. "The code of Robo receptors is what specifies lateral position," Goodman says.

Within a zone, axons respond to more specific cues. For example, axons that express a protein called Fas II are attracted to other axons that express the same protein. Nearly 2 decades ago, Goodman and several colleagues observed that axons send out cell extensions called filopodia toward several bundles of axons, in an effort to find the correct path. However, several sets of axon bundles express Fas II, and no one could explain how an axon distinguishes one Fas II bundle from another.

The Robo-Slit system seems to be the answer. Fas II axons that express just Robo find their way through the midline and join up with the first Fas II neurons they encounter. Those expressing both Robo and Robo3 are pushed by the Slit repellent past the first Fas II bundle and travel outward until they encounter the intermediate bundle. Those expressing all three Robo proteins have such an aversion to Slit that they pass through two Fas II bundles before they are far enough from the midline to join the outermost Fas II bundle.

The model "solves a mystery that goes back 20 years," Goodman says. "The Robo code sends you to a region," and once in the region, "now you go looking for a specific address. The first time you respond to the turn-on of Fas II is when you're far enough from the Slit repellent. Those cues can



CREDIT: JULIE SIMPSON/UC BERKELEY

Robo guidance. The Robo code of receptors helps determine the position of axons (green) in the fruit fly nervous system. The midline produces a protein called Slit (red, upper left), which repels axons expressing Robo (red, upper right), Robo3 (red, lower left), and Robo2 (red, lower right).

be used again in the nervous system, because you'll only pay attention when you're in the right neighborhood."

The explanation "neatly solves several problems," agrees Marc Tessier-Levigne of the University of California, San Francisco, who has studied the Robo-Slit system in mammals. Several researchers who investigate organisms closer to humans—zebrafish, birds, and mice—say the findings may help them discern similar patterns in those animals, as well as in humans. Mammals, at least, have several Slit proteins, plus a suite of Robo family members.

"The model is beautiful, but it's likely to be more complicated in vertebrates," says neurobiologist Chi-Bin Chien of the University of Utah in Salt Lake City, who studies Slits and Robos in zebrafish. Developmental neuroscientist John Kuwada of the University of Michigan, Ann Arbor, who also works with zebrafish, agrees. "Clearly, the Robo-Slit system does work in a somewhat analogous fashion in the vertebrate. Whether it's going to work in this fancy gradient system isn't certain," says Kuwada.

The Goodman team is now working on experiments to find out how the Robo-Slit system helps to control other areas of development. Robo2 is found in the heart, trachea, and muscles of developing flies, and the teams suspect the suite of proteins may provide similar direction there. "Biology uses everything it can: long-range attractants to get to neighborhoods, different local adhesion molecules" to guide more subtle decisions, Goodman says. "All of that together adds up to give precise guidance."

–GRETCHEN VOGEL

MOLECULAR CELL BIOLOGY

A Powerhouse Rises in Reborn Dresden

Two years in the making, a new Max Planck Institute is about to open its doors to scores of talented scientists—many from rival Heidelberg

BERLIN—Devastated by firebombing during World War II and blocked from receiving TV signals from the West during much of the Cold War, the eastern German city of Dresden once bore the unfortunate nickname of "Valley of the Clueless." Now, after a decade of revival, the Saxon capital is positioning itself as a post-communist Silicon Valley. And it is mounting a challenge to one of western Germany's scientific powerhouses in a fast-moving research area: molecular cell biology.

Next month, the Max Planck Society's Institute of Molecular Cell Biology and Genetics will open its glassy new headquarters in Dresden. About a third of the 150 cell biologists, biophysicists, and technicians already hired for the new center have been drawn from the European Molecular Biology Laboratory (EMBL) in Heidelberg, the hub of molecular and cell biology research in Europe for a quarter-century. EMBL's cell biology program has been making up the long-anticipated losses by recruiting from other top cell biology centers in Europe and striking off in new directions.

The Max Planck Society tends to form new institutes around dynamic individuals, and the Dresden institute's catalyst is Kai Simons. The society recruited him in 1998 from EMBL, where he had played a key role in expanding the cell biology program in the 2 decades after arriving from Finland in 1975.

Max Planck chose to locate the institute in Dresden as part of its wave of expansion into eastern Germany in the 1990s. Construction began early last year near Dresden's Technical University, which is building its own molecular bioengineering center. Work is also scheduled to start soon on a privately funded center for bioinformatics. "We're building up an interesting new environment in Dresden," says Simons.

His new institute will probe the way cells organize into tissues. Using time-tested organisms such as fruit flies, nematodes, zebrafish, and mice, researchers will delve into topics ranging from cell division and membrane traffic to cytoskeletal organization and signal transduction. The institute will offer a training program to attract leading Ph.D. students and postdocs, particularly those from countries to the east.

Simons "will pull in a lot of talented researchers from Eastern Europe," predicts EMBL alumnus Graham Warren, a cell biologist at Yale University School of Medicine. "The biggest problem," he adds, "may be in convincing top postdocs from the West to go to Dresden," which is relatively isolated from Western Europe's traditional centers of cell biology research.

Where east meets west. New cell biology institute expects to lure eastern talent.

CREDIT: MAX PLANCK INSTITUTE

A formidable team is already taking shape in Dresden. "Kai's strength is that he can identify not only the best established cell biologists, but also the talented young people who will rise quickly," says Ari Helenius, a cell biologist at Zurich's ETH Polytechnic who worked with Simons at EMBL. Top recruits include former EMBL compatriots Anthony Hyman and Marino Zerial, plus Heidelberg University's Wieland Huttner.

Simons has also demonstrated an appeal beyond Heidelberg, hiring, for example, biophysicist Joe Howard of the University of Washington, Seattle, who studies the mechanics of motor proteins. Simons hopes to have about 300 staffers and limited-tenure researchers in his center's 25 research groups within a couple of years.

EMBL, meanwhile, has not stood still in defending its position among a pantheon of top European cell biology research centers, which includes the Imperial Cancer Research Fund (ICRF) in London and the Marie Curie and Pasteur Institutes in Paris. Eric Karsenti, Simons's successor as coordinator of EMBL's cell biology and cell biophysics program, has hired away from the ICRF Ranier Pepperkok and Philippe Bastiaens, who were joined at EMBL earlier this month by another ICRF alumnus, Damian Brunner.

The restructured program's dozen research groups, Karsenti says, will focus on signal transduction, the cytoskeleton, and membrane trafficking. "We're getting applications from top-notch scientists," he notes.

Yale's Warren agrees that EMBL "needs to identify some young and dynamic researchers and give them the chance to do great research." Some of these young guns may end up heading east, perhaps crossing paths with colleagues in Dresden who get wanderlust and seek their fortunes at EMBL and other centers to the west.

Simons says he would welcome his center becoming part of a molecular cell biology "trade route": "This is exactly what Europe needs," he points out. "Movement."

–ROBERT KOENIG