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Chemical Scissors May Fine-Tune Nerve Cell Migration

Experiments with cultured neurons have hinted that nerve cells may actually use a chemical scissors to snip off a portion of an axon guidance receptor in order to ensure proper navigation in the central and peripheral nervous systems.

The research provides another tantalizing hint of how axons—the growing tips of neurons—make decisions about where to migrate in order to wire up the developing nervous system. "We've known for several years that neurons have to change their preferences as they move along," said Howard Hughes Medical Institute (HHMI) investigator Marc Tessier-Lavigne. "As neurons grow, they extend their axon first to an intermediate target that they find attractive. But once there, they must lose the attractive response, or they would stay there forever. What once was attractive must now become neutral, or even repulsive."

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— Marc Tessier-Lavigne

In the experiments, which were reported in the August 25, 2000, issue of the journal *Science*, Tessier-Lavigne and University of California, San Francisco colleague Michael J. Galko showed that cultured neurons alter their migration toward an attractive signal by selectively clipping off a portion of a receptor that protrudes from the axon's surface. In this case, the attractive signal is provided by the protein, netrin-1, and the clipped receptor is the protein, DCC, a netrin receptor. Netrins are proteins that have been shown to attract axons.

If further experiments support this new model of axon guidance, the discovery may stand as a third mechanism by which axons can control their progress in the developing nervous system. Tessier-Lavigne and others had demonstrated that axons can alter their progress by increasing or decreasing the number of netrin receptors on their surfaces and by modulating internal

signaling when a netrin receptor is activated.

Tessier-Lavigne described his group's *in vitro* studies of netrin-1 receptor cleavage as a proof-of-principle experiment. "We have shown that this type of control mechanism can operate *in vitro*," he said. "Now we must find out whether it operates in the living organism."

The scientists' experiments began as an attempt to isolate a brain protein that enhanced the sensitivity of axons to netrins. As they started their search for the NSA (netrin-synergizing activity) protein, Tessier-Lavigne and Gallo had a hunch that NSA might be a protein that functions to inhibit a metalloprotease, an enzyme that snips apart other proteins. They based this hunch on biochemical studies that suggested, but did not prove, that NSA would be a metalloprotease inhibitor. They were intrigued by the possible involvement of a metalloprotease because *Drosophila* studies by HHMI investigators Corey Goodman and Gerald Rubin (who is now HHMI vice president for biomedical research) showed that a mutation in a fruit fly metalloprotease gene called *kuzbanian* stalled the growth of axons.

"While we couldn't fully pin down the identity of NSA, we reasoned that we should test the effects of chemical inhibitors of metalloproteases on netrin sensitivity," Tessier-Lavigne said. "Lo and behold, we found that two chemically distinct inhibitors of metalloproteases could, indeed, potentiate netrin activity."

In an attempt to pinpoint exactly how metalloprotease inhibitors could increase netrin activity, Tessier-Lavigne and Gallo looked at the effects the inhibitors had on DCC, the netrin receptor. "It was known that many surface receptors are cleaved by metalloproteases," said Tessier-Lavigne. "We thought that if, by chance, DCC was cleaved, then maybe that cleavage would be arrested by a metalloprotease inhibitor, and maybe that would make the cell more responsive to netrin."

Indeed, the researchers' analyses of cultured neurons provided two interesting results. "First, we showed that DCC is normally cleaved and shed from cell surfaces," said Tessier-Lavigne. "Second, we found that the metalloprotease inhibitors did block this cleavage." The scientists also demonstrated that two inhibitors that mimicked the action of NSA specifically blocked netrin-receptor cleavage *in vitro*, while a third inhibitor that was unlike NSA did not show the same effect.

"There is still one loose end," says Tessier-Lavigne. "We would expect NSA to behave as an inhibitor of DCC cleavage, but in fact it doesn't. It seems to produce its effect by doing something else, perhaps blocking the cleavage of another protein involved in the netrin receptor system."

According to Tessier-Lavigne, future work will concentrate on isolating the NSA proteins and identifying how NSA alters DCC. "We also hope to be able to pull the whole story together by finding some place in an organism where this mechanism is actually used to regulate axonal decision-making," he said. "We suspect that the metalloprotease mechanism will be used in a

situation where neurons are responsive to a factor such as netrin until they've grown to a particular region. And then the protease will clip off the receptors so the axon stops becoming responsive and can move on. But this is still a working hypothesis."