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## Brain-Wiring Receptor Shows Extraordinary Diversity

Researchers have identified a new axon guidance receptor found in the tips of growing neurons that can exist in more than 38,000 slightly different forms.

The unprecedented diversity of proteins derived from this single gene may offer an important hint that a fundamental code directs the precise wiring of trillions of neurons in the brain.

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— S. Lawrence Zipursky

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In an article in the June 2000 issue of *Cell*, researchers in the laboratories of HHMI investigator S. Lawrence Zipursky at the University of California, Los Angeles (UCLA) and Jack Dixon at the University of Michigan report that the *Drosophila* protein Dscam is an axon guidance receptor. Dscam is the fly version of the human protein DSCAM (Down syndrome cell adhesion molecule). Axon guidance receptors are nestled in the membrane of axons, the projections of neurons that are guided toward their targets in the central and peripheral nervous systems by attractive and repulsive cues.

After Zipursky and his UCLA colleagues, Dietmar Schmucker, James C. Clemens, Huidy Shu and Jian Xiao, showed that Dscam is an axon guidance receptor, they performed detailed molecular genetic analyses of Dscam. Their studies showed that the Dscam messenger RNA is processed in such a way that it can produce a huge number of slightly different proteins. By the researchers' calculations, alternative splicing of the Dscam messenger RNA could generate more than 38,000 variations, or protein isoforms. "These proteins would all have the same overall structure, but they would have different amino acid sequences in some of the domains," said Zipursky. Initial experiments by Zipursky's group indicate that a large number of these Dscam isoforms are, indeed, present in fly embryos.

"We were quite surprised by these results," said Zipursky. "They came right out of the blue. This is a spectacular diversity. Although we're at a very early stage of exploring this phenomenon, it suggests that these related molecules may provide some sort of code by which neurons achieve their extraordinary specificity."

Zipursky and his colleagues embarked on their studies initially seeking to refine their characterization of the internal signaling pathways that guide axons to their proper destination. Earlier studies by the team had revealed that the protein Dock acts as an adapter inside the axon that links an unknown receptor in the axon membrane to a signaling protein called Pak. Pak, in turn, generates signals that control the motility machinery in the extending axon.

To attempt to identify the unknown receptor, Clemens, while a graduate student in Dixon's laboratory at the University of Michigan, launched a search for proteins that attached to Dock in a location other than that occupied by Pak. He found one protein that fit the search criteria. Further analysis revealed that the *Drosophila* protein was similar to a human protein of unknown function, DSCAM, which had been hypothesized to play a role in abnormalities in brain development linked to Down syndrome. Due to the similarity between the two proteins, Zipursky's group named the newly identified fly protein, Dscam, although Zipursky emphasizes that any implications of the fly studies for human Down syndrome are "completely unclear."

Further studies on Dscam showed that it physically interacted with Dock inside the cell and was a functional component of the *Drosophila* axon guidance pathway.

In one such study, Schmucker, who is an HHMI associate, explored the effects of Dscam malfunction on growing neurons in Bolwig's nerve, an easily studied sensing organ found in fly larvae.

"The Bolwig's nerve is an incredibly simple structure for studying axon guidance, with only twelve neurons in a single bundle," explained Zipursky. "In contrast, the adult fly visual system has eight hundred different bundles, all growing into the brain. Trying to sort out the mistakes in guidance that can occur in such a system is like arriving at a traffic pileup on a freeway and trying to figure out what happened."

Schmucker's studies showed that mutant forms of Dscam on Bolwig's nerve disrupt axon guidance and cause significant mistargeting of axons.

The major surprise came when the researchers began to look for alternate versions of Dscam. They found no evidence of variability in the segment of the Dscam protein that is anchored to the cell's interior. They did, however, find considerable variability in the segment of the molecule that extends outside the cell.

"We weren't prepared for that," said Zipursky. "Fortunately, just as we were discovering these multiple forms in cDNAs, the sequence of that entire region

of the fly genome became available, so within a few days we were able to sort out the source of the multiple forms."

Using the *Drosophila* Sequencing Project's data, the scientists analyzed the different regions of the *Dscam* gene and found that it had the potential to express more than 38,000 versions of Dscam.

In a small study of 50 randomly chosen cDNAs, 49 were found to be different, indicating that such alternative versions of Dscam are likely used in the developing fly brain.

According to Zipursky, further research may reveal how such massive diversity of axon guidance receptors aids in the wiring of the fruit fly brain.

"The important remaining questions include whether these different forms are expressed in different neurons, or perhaps in the same neurons at different times, and whether they have distinct functions," he said. "We don't know the answer to those questions yet." Zipursky also emphasized that since no comparable diversity of axon guidance receptors had been found in humans or other mammals, further research is needed if the findings in flies are to be extended to other organisms.