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Gene-Trapping Method Powers Discovery of New Brain-Wiring Signals

Researchers have developed a powerful screening method to identify genes that produce proteins that guide the wiring of the trillions of connections in the mammalian brain. The technique enables scientists to identify new genes and to determine which genes are responsible for defects in brain wiring that are observed during development. The scientists believe that this technique is likely to accelerate the discovery of new molecules involved in axon guidance.

Neurons wire themselves into networks by extending cable-like axons that grow toward specific targets in the nervous system. An axon's path toward a target neuron is steered by growth cones in the tip of the axon that receive cues about the best path to follow from chemical attractants and repellents secreted by cells in the nervous system. These attractants and repellents are collectively called axon guidance molecules.

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— **Marc Tessier-Lavigne**

In an article published in the March 8, 2001, issue of *Nature*, researchers led by Howard Hughes Medical Institute investigator Marc Tessier-Lavigne at the University of California, San Francisco and William C. Skarnes at the University of California, Berkeley, unveil their new technique and discuss some early applications of the method.

The new "gene-trapping" technique could liberate scientists from laborious genetic screens and biochemical approaches that are currently used to identify

new molecules involved in axon guidance, the researchers say. “Up until now, we’ve gone about trying to identify brain wiring mechanisms one guidance event at a time, one molecule at a time,” said Tessier-Lavigne. “For example, in past work studying axon guidance in the spinal cord, we developed an assay to study the growth of one particular class of axon. And that study then led us, through extensive biochemical work, to identify a small family of guidance molecules called the netrins.”

Guidance molecules either attract or repel the growing axons of neurons by plugging into receptors on the surface of the axon tip. Typically, each guidance molecule or receptor is identified individually through time-consuming screening of random chemically induced mutations, Tessier-Lavigne said. “However, with the gene-trapping approach, we can cast a much wider net, studying a great many genes simultaneously, and then determining the effects of mutating them.”

The gene trapping technique was built on a method developed earlier by Skarnes at the University of California, Berkeley. Skarnes’ technique involved mutating genes in mouse embryonic stem cells by randomly inserting a complex genetic marker with two components—the first is a marker gene that produces a blue color in cells carrying the inserted gene and the second is a drug-resistance gene. Thus, the scientists can easily identify cells of interest by applying a drug to weed out those that did not take up the drug-resistance gene and then use the blue color to distinguish them further.

Skarnes’s method refined this standard “gene-trap vector” to include a gene segment that would only activate the blue marker if the DNA had fused itself into a gene for a membrane protein, such as a receptor. With this refinement, called a “secretory trap,” the researchers were able to narrow down the trapped genes to those coding for receptors of the kind involved in axon guidance.

“The secretory trap vector is a nice bonus because we can focus on exactly the kinds of molecules we’re interested in—mainly receptors and ligands,” said Tessier-Lavigne. “These genes represent only a small fraction of the genome, and this trap concentrates on just that fraction.”

However, the gene trap still needed further refinement before it was ready for use in fishing out axon guidance molecules, said Tessier-Lavigne. “In early studies, we found that mice with ‘trapped’ neuronal genes didn’t show proper axon staining,” he said. Thus, the researchers had a difficult time exploring the effects that specific gene mutations had on brain wiring.

In trying to fix the problem, Tessier-Lavigne and his colleagues inserted an additional marker (PLAP) in the gene-trap system. The presence of PLAP stains axons purple. “This modified gene-trap strategy enabled us to mutate a gene for a guidance molecule receptor, and by including the PLAP marker, we were able to see the purple-stained altered neuronal wiring and rapidly assess what has gone wrong with the wiring process,” said Tessier-Lavigne.

Using the modified gene-trapping technique, the researchers produced 46 lines of mice with defined defects in axon guidance molecules, said Tessier-Lavigne. “With these mice, not only have we proven that we can trap genes that are specifically expressed in the nervous system, but we can also see discrete patterns of axonal labeling, and we can uncover mutant phenotypes,” he said.

Specifically, studies on genes, called *Sema6A* and *EphA4*, demonstrated that the trapping method could identify axon guidance mutants.

“With *EphA4*, we showed that we could re-derive a known mutant, and with *Sema6A* we showed that we could use the technique to discover a new mutant that affects only a small subset of axons in an otherwise normal nervous system,” said Tessier-Lavigne.

These results suggest that the new gene-trapping method will enable a rapid increase in understanding the strategy neurons use in wiring the developing brain.

“It has been shown that neurons that project their axons to a particular area follow a code of transcription factor activation that presumably activates genes for surface receptors that, in turn, dictate what the axon does,” said Tessier-Lavigne. “We’re hoping that this method can help identify the underlying code by focusing very specifically on receptors involved in axon guidance and finding their expression patterns as well as their mutant phenotypes.”

Furthermore, the mutant mouse lines produced by this technique should also aid attempts to map the normal wiring of the brain. “These mouse lines have very specific populations of axons that are labeled purple,” said Tessier-Lavigne. “In some cases it’s the first time that a marker has been identified for those axons, and those markers provide a valuable resource for people who want to study the normal wiring pattern of the brain.