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## Controlling the Synapse--49 Proteins at a Time

In the exquisitely regulated networks of the brain, hundreds of channels, receptors, and other specialized proteins work together to control communication at the synapses, or junctions between neurons. Working with mice, scientists have found that a single molecule, known as Nova, helps control the production of a large, closely related set of these specialized proteins.

The 49 proteins regulated by Nova all play some role at the synapse - as neurotransmitter receptors, ion channels, adhesion molecules, and scaffold proteins - or in guiding the development of axons, the long projections of the nerve cell body. According to the researchers, Nova's role in controlling the production of these related proteins represents a means by which the entire set can be regulated as a group.

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“What we found is that Nova regulates a very refined, restricted piece of biology, and it regulates it in a very multi-tiered and complicated way,” said Robert B. Darnell, a Howard Hughes Medical Institute investigator at The Rockefeller University and senior author on the study. Darnell's colleagues at Rockefeller and scientists from the University of Amsterdam, Affymetrix, Inc., and the National Cancer Institute also contributed to the work, which was published in the August issue of the journal *Nature Genetics* .

Like all cells in the body, brain cells must rely on the same limited set of genes - about 20,000 to 30,000 - as the blueprint for the unique assortment of proteins they need to carry out their function. One way cells achieve this diversity is by modifying messenger RNA molecules - the intermediary material in the conversion of gene to protein. Regulatory molecules like Nova oversee the cutting and pasting of the RNA - including and excising different bits and pieces in a process known as alternative splicing - to produce an

assortment of proteins from the same gene.

Several years ago, Darnell and colleagues discovered that Nova is a key player in regulating alternative splicing of RNA molecules in the brain. The team initially identified Nova because it was the target of autoimmune attack in an uncommon neurological disorder known as paraneoplastic opsoclonus myoclonus ataxia, which causes progressive loss of motor control. Further investigation revealed that the protein, which is found only in the brain, bound to RNA molecules and regulated their splicing.

It was clear that Nova was not needed for all of the alternative splicing that occurred in the brain, but was instead involved in the splicing of only a specific set of RNA molecules. "What we wanted to ask was 'Is there a functional coherence among the Nova targets?'" said Jernej Ule, a postdoctoral in Darnell's laboratory who is the first author on the study. Preliminary evidence suggested that there was - of the 10 known Nova target genes, most produced proteins with a role at the synapse. Only a genome-wide screen for Nova targets, however, would tell them for sure.

To zero in on the specific gene products that Nova regulates, the researchers used microarray technology, which is typically used to measure differences in levels of gene expression. For this study, the team used a new microarray specially designed to detect variations in gene splicing.

A cell's splicing machinery, known as the spliceosome, can snip an RNA molecule only at certain locations, or splice junctions - meaning there is a finite collection of RNA sequences that might exist where those junctions are rejoined. The team used a microarray from Affymetrix that was custom designed to detect the alternative splicing of more than 8,000 alternative protein forms produced from approximately 3,000 mouse genes.

By comparing the RNA molecules found in specific regions of the brain with those in parts of the immune system, the researchers found more than 570 genes whose products were spliced differently in the brain.

The researchers then used the microarray to compare how genes were spliced in the brains of normal mice and in mice that lacked Nova. They found 49 transcripts that were spliced differently in the absence of Nova, confirming each of these with biochemical studies. Focusing on one defined region of the brain, the neocortex, the scientists found that Nova contributed to seven percent of the brain-specific splicing that occurred there.

Knowing that Nova's control over splicing focused on a narrow subset of the total group of genes that are alternatively spliced in the brain, the researchers set out to investigate whether these genes shared a common function. "We found that there is an incredibly significant overrepresentation of synaptic genes among the Nova targets. We found almost no other function. The kind of coherence we got is really unprecedented," Ule said.

They found that 85 percent of the genes regulated by Nova were involved in communication at the synapse, and 20 percent were involved in guiding the

axon to the appropriate target in the brain during development. In contrast, the overall set of genes that undergo tissue-specific splicing in the brain includes “a little bit of everything,” Darnell said.

Not only do Nova targets share similar functions, the researchers said, but the majority of those proteins interact with one another. “This means that by regulating one protein - Nova,” Darnell said, “what is being regulated is the quality of the proteins and the quality of the protein-protein interactions that go on at the synapse.”

“Any regulation through Nova would be able to affect the function of these genes in a very coherent manner,” Ule noted. It's not yet known how Nova itself is regulated, but the lab has begun to investigate this question, as well as under what conditions Nova regulation may be important. Because of its actions on synaptic proteins, Nova may play a role in normal processes such as memory, they say, while understanding its regulation may be of importance in neurologic disorders that involve the synapse, such as epilepsy.