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Map Helps Explain Alternative Splicing in the Brain

Combining data from years of laboratory work with the power of bioinformatics, researchers have created a map that helps explain how the brain generates the assortment of specialized proteins it needs to process information.

The map, created by Howard Hughes Medical Institute (HHMI) investigator Robert B. Darnell and colleagues at The Rockefeller University, describes the rules that govern the activity of a protein called Nova. By regulating a process called alternative splicing, Nova helps brain cells produce a set of proteins involved in communication at synapses, or the junctions between neurons. The new study, published October 25, 2006, in an advance online publication of the journal *Nature*, furthers understanding of a process that, when not properly regulated, can lead to cancer, neurologic diseases, or other ailments.

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— **Robert B. Darnell**

Limited to the same set of genes that encode the instructions for all cells in the body, brain cells rely heavily on alternative splicing to generate the protein diversity they need to function properly. The process, at work in all cells in organisms ranging from fruit flies to humans, chooses bits and pieces of an RNA copy of a gene, piecing segments together to form a blueprint for the precise protein that is needed. Using alternative splicing to assemble different patterns, a single gene can give rise to multiple — sometimes thousands — of proteins.

Scientific interest in alternative splicing has grown in recent years, in part because the phenomenon helps explain how humans can be so complex despite having a genome that is surprisingly similar in size to that of simpler organisms, like flies and worms. It's a way that orders of magnitude of complexity can be layered on to the 20,000 genes that we have, Darnell said. At the same time, alternative splicing is also becoming increasingly recognized as playing a role in disease, especially neurologic disease. For

example, alternative splicing errors are known to contribute to cystic fibrosis, growth deficiencies, and neurologic disorders including ataxia, autism, and muscular dystrophy.

In the genes of most organisms, the instructions for building a protein are interrupted by segments of DNA known as introns. When the conversion of gene to protein begins, introns are copied into a pre-messenger RNA along with protein-coding segments of DNA, known as exons. Before the pre-messenger RNA is used as a template for protein production, however, the cell removes these interrupting sequences to generate the mature messenger RNA that is used as the final template to produce proteins. In genes that undergo alternative splicing - for example, more than 50 percent of human genes - certain exons can also be removed during this process. Whether an exon is included may vary in different cell types or under changing conditions, ultimately altering the protein that is produced.

To ensure that the correct exons are included or excised from each RNA molecule, regulatory molecules like Nova oversee the alternative splicing process. Evidence of Nova's critical role in the nervous system can be seen in patients who have a defect in the Nova protein. Those patients develop paraneoplastic neurologic syndromes, a focus of study in Darnell's lab. Patients with the defect seem to have a special problem with inhibiting neurotransmission - they have too much motor movement, too much eye movement, Darnell explained. Mice that lack Nova, created in Darnell's lab, have the same problems.

While it's clear that alternative splicing normally acts under tight control, with dozens of splicing factors playing a role, Darnell said little is known about the rules governing how these regulators identify an alternative exon and tell cells whether or not to include it. He noted that even less is known about how this works in organs such as the human brain.

In the case of Nova, Darnell's lab had shown previously that the protein zeroed in on sites on the RNA molecule that were marked by a characteristic sequence, which they called a YCAY cluster. But that same sequence caused Nova to promote the splicing of some exons, and inhibit the splicing of others. Spotting a YCAY cluster on any RNA suggested that Nova helped regulate its splicing, but there was no way to know whether Nova would turn splicing on or off. Darnell said similar effects had been observed with other splicing factors in studies with laboratory-grown cells.

Darnell and his colleagues originally identified the YCAY clusters by studying three Nova-regulated RNA molecules. To truly understand how Nova functioned, they needed to look at a larger collection of molecules. In a study published in 2005, the team had compared alternatively spliced exons in the RNA of mice with and without Nova, and turned up 50 RNA targets whose splicing was affected by Nova.

Taking a closer look at these, Jernej Ule, a graduate student and then postdoctoral fellow in the Darnell lab, found that indeed, like the three original Nova targets, these molecules included YCAY clusters. Furthermore,

Darnell said, we found an absolute correlation between the transcripts where Nova enhanced exon inclusion versus inhibited exon inclusion and the location of these YCAY clusters. It turned out, Darnell said, that if the YCAY cluster lay inside an alternatively spliced exon, or in the intron in front of it, Nova triggered the skipping of that exon in the final messenger RNA. If, however, the YCAY cluster lay in an intron downstream of the regulated exon, Nova would promote its inclusion. With these rules, the researchers were able to create an RNA map - describing how Nova should behave based on the features of the RNA.

The next step was to see if these rules applied to RNA molecules the lab had not yet studied. If we looked blindly at all of the alternatively spliced transcripts in the brain and searched for those that have clusters of Nova-elements, could we predict whether they are regulated by Nova? Of those that are, do they fit the map? Darnell said. To find out, the team turned to a large database of alternatively spliced exons created by Rockefeller colleague Terry Gaasterland, and searched for YCAY clusters. Once they had identified and validated about 25 Nova targets, they found that all of these were in fact regulated by Nova precisely according to the rules of their map.

Adding biochemical studies to their investigation, Giovanni Stefani, also a graduate student and then postdoctoral fellow in the Darnell lab, went on to show that Nova's effects on alternative splicing were due to its ability to physically block or promote - depending on its position on or near an alternatively spliced exon - the assembly of the large protein complex called the spliceosome, which carries out RNA splicing. The RNA map tells us that there's an asymmetry in how Nova is binding and that asymmetry determines the splicing outcome, Darnell noted.

The map that we identified by laying bioinformatics on top of biochemistry and genetics gave us a global view of how Nova is working, Darnell said. For the first time we are able to develop an understanding of how a splicing factor works on its full array of targets. He added that beyond the new insight into Nova's function, the study suggests a strategy for detailed study of other splicing factors, to better understand both the basic biology of the process and the many diseases with which they are linked.