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Technique May Identify Novel Disease Genes at a Faster Clip

Researchers have used ultraviolet light to “weld” a key regulatory protein to its RNA targets, creating a new tool that can be used to identify novel proteins involved in a variety of human diseases.

Using this technique, the researchers have identified an array of RNA molecules regulated by the RNA-binding protein, Nova, which has been implicated in an autoimmune neurodegenerative disease. The researchers believe their technique may help in finding the RNA targets of other proteins involved in neurological diseases, including the most prevalent form of mental retardation, the Fragile X syndrome.

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Robert B. Darnell, a Howard Hughes Medical Institute investigator at The Rockefeller University, led the research team that reported its findings in the November 14, 2003, issue of the journal *Science*.

Darnell and his colleagues have been investigating the function of Nova, an RNA-binding protein that regulates alternative splicing. In alternative splicing, messenger RNA, carrying the blueprint for a protein from the cell's genes, is processed in such a way that it can produce a number of slightly different proteins. Apart from its role in alternative splicing, the Nova protein is of great interest, said Darnell, because it is targeted by the immune system in the neurodegenerative disease, paraneoplastic opsoclonus myoclonus ataxia, which causes progressive loss of motor control.

“Previous work in our laboratory had revealed how Nova bound to RNA, and we had identified a couple of specific target RNAs in the brain,” said Darnell. “These studies led us to discover that Nova was the first mammalian splicing factor that was restricted to a particular tissue. We then really wanted to know, What is the full array of RNAs that Nova binds to and regulates in the

brain?”

According to Darnell, Nova is just one of a rapidly growing list of RNA-binding proteins that are being implicated in human diseases. Thus, a technique that can help identify the multiple RNA targets regulated by an RNA-binding protein could potentially aid in understanding the cause of many human diseases.

To facilitate identification of the target proteins, the scientists adapted a technique that had been used in the test tube to identify the targets of RNA-binding proteins. This technique involved irradiating molecules with ultraviolet light, which caused a cross-linking reaction that chemically bonded the protein with its RNA target. The bond is so tight that the molecules could be isolated and identified together.

Darnell and his colleagues made some enhancements that resulted in the development of their “cross-linking and immunoprecipitation” (CLIP) technique. The researchers began by irradiating intact mouse brains with UV light, seeking to weld together RNA-binding proteins and their RNA targets in living tissue. Following a technically demanding purification and analytical procedure, the researchers were able to pinpoint some 340 Nova CLIP “tags”—telltale pieces of Nova-bound RNA that identified the RNA target molecule and revealed where the Nova protein bound to it. The researchers verified that the tags represented functional Nova RNA targets by comparing their splicing in wild-type mice with knockout mice lacking Nova.

The striking splicing changes in the knockout mice, said Darnell, constituted proof that Nova is the central regulator of splicing in a whole set of RNA molecules found in the brain. “We’re finding that Nova is an extremely important factor—maybe *the* factor—that is responsible for neuronal splicing for some targets,” he said.

Their studies turned up another important observation: Nova does not act randomly. “Looking at these targets as a group, they have a tremendous biological coherence to them,” said Darnell. “Almost seventy percent of them are RNAs that have something to do with the neuronal synapse.” Synapses are the junctions between neurons. One third of the Nova synaptic RNA targets encode proteins involved in inhibiting neuronal function. Regulating neuronal inhibition plays a key role in the balance controlling nervous system function normally as well as in neurologic disorders such as epilepsy, said Darnell.

“These findings suggest that Nova has evolved to regulate a set of RNAs that have a coordinate function,” he said. “So, if you turn Nova function up or down, you’ll coordinately regulate a group of RNAs en masse.”

The success of the CLIP method in identifying Nova targets, said Darnell, suggests that it will find broad use in discovering targets of other RNA-binding proteins, including those involved in such diseases as Fragile X mental retardation. “The study of the fragile-X-syndrome protein has been stuck, because knowing it’s an RNA-binding protein doesn’t really tell you

what it's doing," said Darnell. "The problem has been to identify the set of RNAs that it regulates. We and others have made some progress using other techniques, but CLIP should help solve this problem."

CLIP has also revealed that Nova may play a previously unsuspected role besides regulating alternate splicing. "We found quite a few instances of CLIP tags that are not near alternative splice sites, but are at the beginning or end of an RNA," said Darnell. "This suggests that there may be some brand new biology going on that we didn't suspect." This new form of regulation might be occurring as RNA's information is being translated into a protein by the cell's protein-making machinery, Darnell said.