

JUNE 17, 2009

New Strategy Rapidly Reveals Targets for MicroRNA Gene Regulation

A new method promises to cut through the stubborn problem of determining the precise targets of microRNAs – the tiny but powerful bits of nucleic acid that tweak gene expression to influence many aspects of health and human disease, from early development and aging to cancer, heart disease, and diabetes.

Researchers using the new technique, called HITS-CLIP, showed that in a single experiment they could map the binding points of scores of different microRNAs throughout a genome in living mouse or human tissue. The research by Howard Hughes Medical Institute investigator Robert Darnell and his colleagues Sung Wook Chi, Julie Zang, and Aldo Mele at The Rockefeller University was reported June 17, 2009, in an advanced online publication of the journal *Nature*.

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— **Robert B. Darnell**

Darnell believes that in speeding the mapping of microRNAs to their targets, the technique can alleviate a bottleneck in this burgeoning area of RNA research. His team has already used the approach identify the targets and binding sites of nearly 90 percent of the microRNAs that regulate gene expression in the mouse brain. "To a first approximation, we can map every site they bind to on every RNA transcript," he says. Information gleaned from HITS-CLIP mapping could be helpful in learning how microRNAs influence the progression of diseases, and in identifying targets for new drug therapies, Darnell says.

RNA, a nucleic acid, is still best known for its role as a messenger, relaying instructions from the DNA of genes to parts of the cell that make proteins. MicroRNAs are short stretches of 20 to 22 nucleotides that do not encode instructions for making a protein. They regulate gene expression by blocking the production of specific proteins. These snippets of RNA do their work in the cell's cytoplasm, where they intercept messenger RNA that carries a gene's blueprint to the ribosome factories where proteins are made.

MicroRNAs dock onto a binding site on messenger RNA. The interaction shortens the lifetime of the messenger RNA or blocks or weakens its instructions, thus reducing the output of protein.

Human genes are subject to the control of more than 500 different microRNAs – collectively thought to regulate more than half of our genes. And hundreds of microRNAs have been identified in other species, as well. To understand their full impact, researchers would like to have a comprehensive catalog of which genes are

targeted by each microRNA, as well as where on each messenger RNA molecule that interaction occurs. Until now, however, there has been no efficient way of determining that information.

“To know what a particular microRNA is doing in a cell you need to know what messenger RNA it is binding to, and exactly where it binds,” says Darnell. But “the field hit a roadblock in finding out what any given microRNA does.”

That’s because microRNAs latch on to their target transcript with as few as six consecutive nucleotides. That six-nucleotide sequence might occur many times within a single messenger RNA, and finding the actual minuscule “footprint” is a technical challenge. The problem, Darnell explains, is that the messenger RNA “is like a long coastline with a lot of ports, and it’s been very difficult to know where the microRNA docks.” Biochemical and computer prediction methods are imperfect, he says, and so the tedious hunt for binding sites could only be done experimentally, one RNA at a time.

To get a clearer view of where microRNAs interact with messenger RNAs, Darnell and his colleagues used a technique to study protein-RNA interactions in living, intact cells. HITS-CLIP, which stands for high-throughput sequencing – cross linking immunoprecipitation, was first developed by the Darnell lab in 2008, and is an improvement on their development of CLIP in 2003. CLIP relies on an old laboratory trick that essentially “freezes protein-RNA complexes inside the living cell,” Darnell says.

To freeze these complexes, the researchers shine ultraviolet light onto the cells they are studying, which causes all the proteins and RNAs that are very close to each other become cross-linked – they form an unbreakable bond. Applying HITS-CLIP to a protein called Argonaute turned out to be an effective way of revealing microRNA-messenger RNA interactions. Argonaute assists the regulatory activity of microRNAs by latching onto a microRNA and guiding it, like a tugboat, to its dock on the messenger RNA. Ultraviolet crosslinking locks Argonaute into a complex with both the microRNAs and its messenger RNA target.

The cells can be broken open with a strong detergent that washes away everything but tightly bound RNAs and proteins. “Then we get rid of the proteins,” says Darnell, “and we’re left with just the microRNA and a tag of the Argonaute protein bound to the

messenger RNA.” Sequencing those bits of genetic material reveals where Argonaute was bound to both the microRNA and the messenger RNA.

When those data sets are crunched with bioinformatic algorithms, what falls out is the tiny “footprint” on the messenger RNA where the microRNA attaches. “With high-throughput sequencing, we can instantly see all the microRNA binding sites on all the messenger RNAs,” Darnell says.

His lab is interested in human brain disorders, and they have been using HITS-CLIP to study how genes are regulated in brain tissue. They first applied HITS-CLIP to the set of microRNAs in the mouse brain and identified binding sites for about 90 percent of those microRNAs with better accuracy and specificity than computer predictions.

Darnell is excited about the prospect of using his method to glean a new understanding of how microRNAs tweak genes in defined pathways to shape development and other vital processes. He also plans to compare the role of microRNAs in healthy and diseased brains, and expects other researchers will carry out similar experiments to catalog microRNA interactions in other tissues and under different conditions.

“We hope to de-convolute an extremely complicated problem into something that begins to make sense,” he says. “It’s really beautiful.”