More accurate, powerful genetic analysis tool opens new gene-regulation realms

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Researchers from Huntsman Cancer Institute (HCI) at the University of Utah have developed a novel and powerful technique to identify the targets for a group of enzymes called RNA cytosine methyltransferases (RMTs) in human RNA. They applied their technique to a particular RMT, NSUN2, which has been implicated in mental retardation and cancers in humans, finding and validating many previously unknown RMT targets—an indication of the technique's power. The research results were published online in the journal *Nature Biotechnology* on April 21.

"Although RMTs have been known for many years, virtually nothing is known about the majority of these enzymes in humans," said Bradley R. Cairns, co-author of the study and Senior Director of Basic Science at HCI. "This new technique will now allow the very rapid identification of the direct target RNAs for each human RMT, and we are excited about conducting that work."

Within all living cells, RNA acts as a critical intermediate in transmitting genetic information from DNA—RNA is made from DNA and then used to encode proteins called enzymes that control cell functions. A process called cytosine methylation attaches methyl molecules to cytosine bases in DNA and RNA molecules. RMTs act as catalysts to allow methylation at particular locations in RNA molecules. Methylation can regulate the flow of genetic information (from RNA to protein production) in cells, and it can change the way RNA interacts with proteins.

RNA methylation is currently poorly understood, partly because of limitations in the technique currently used to identify which RNA molecules and cytosine bases are RMT targets. As each cell contains thousands of different types of RNA molecules, often with only a small percentage being targets for a specific RMT, the first step in a study of RNA methylation is to sort out and concentrate the precise target RNA molecules for a particular RMT, in a process called enrichment.

The work involved a novel enrichment method, which applied a special "chemical cross-linker" to physically join the RMT to an RNA that it is trying to methylate, said Vahid Khoddami, the study's co-author and a member of the Cairns Lab. "Our new technique takes advantage of the mechanism of the enzyme. The drug/crosslinker we used looks like cytosine, so it is incorporated in place of the cytosine in the RNA. The RMT tries to methylate this drug— thinking it is a normal target cytosine—but instead becomes crosslinked to the RNA, defining the precise location of the intended methylation. As our reaction-based method requires that the enzyme both bind the RNA and commit to the act of methylation, it greatly increases our identification of true positives," said Khoddami.

"This technique gives us 200-fold enrichment, when two-fold enrichment has been considered a great result in the past," said Khoddami. "In fact, for some RNA types, the enrichment is more than 700-fold."

After the enrichment process, high-throughput gene sequencing is used to analyze the RNA samples obtained.

"Our enrichment results were fantastic by themselves, but in the sequencing process we made another important discovery," Khoddami said. "We found that after sequencing, the target cytosine in the modified RNA instead appeared as an alternative molecule, guanosine,

more than 50% of the time. After sequencing, you can look for these cytosine to guanosine transversions and know you have the precise target—in a single experiment."

According to Khoddami, ten [cytosine](#) RMTs are known in humans, and only two of them have been partially characterized. "None of the other eight have been studied in the laboratory," he explained, "although some of them have been shown to have connections to cancer, infertility, and particular genetic disorders in humans.

"These diseases have been puzzling because previously we did not have the tools to analyze the RNA. Now we have beautiful tools," said Khoddami.

Provided by University of Utah Health Sciences

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