Scientists reveal how cells destroy RNA, a key to understanding disease

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(Phys.org) —RNA encodes the proteins that play a key role in cellular reproduction, but the manner in which cells regulate RNA's removal once proteins are synthesized remains a mystery. One piece of this mystery has been solved by researchers at the University of North Carolina at Chapel Hill who have identified the steps by which a cell removes RNA from the cytoplasm.

Understanding the basic function of RNA will help build a greater understanding of the proteins and pathways involved in a host of biological functions that lie at the heart both of normal biological function and the development of genetic diseases such as cancer.

Using high throughput sequencing, a team led by William F. Marzluff, PhD, Kenan Distinguished Professor of biochemistry and biophysics at the UNC School of Medicine and member of the Integrative Program in Biological and Genome Sciences and the UNC Lineberger Comprehensive Cancer Center, analyzed millions of strands of RNA to identify the process by which a cell degrades histone messenger RNAs (mRNAs). The results of the study were published in the journal *Molecular Cell.*

"It is really critical to make histone mRNAs in the right amounts and keep them present in the right amounts. One of the major mechanisms the cell uses to do that is to rapidly get rid of them when DNA synthesis stops. It is important for a cell to do that or the chromatin gets messed up," said Marzluff.

Histone mRNAs manufacture the protein components of chromatin, which together with DNA form the chromosomes in the cell nucleus. Like all RNA, it is comprised of strands of nucleobases, the molecules of adenine, cytosine, guanine, and uracil that provide the "letters" of the genetic code. In mRNA, a molecular machine known as a ribosome reads the order of the nucleotides and uses the information to assemble proteins.

The cell tightly regulates the level of histone mRNAs. During cell reproduction, the level of the mRNA increases 35-fold as DNA is replicated and returns to normal levels as the cell begins to divide. While researchers have understood some of the steps by which the RNA is removed from the cell, Marzluff's team is the first to observe the entire process.

"Degrading the RNA at the right time is as important as making it. The degradation is regulated in the same way that transcription is regulated," said Marzluff. "We can measure the fact that it is being degraded and we have now found all the intermediates along the way."

Histone mRNA degradation begins when a string of uridine molecules are added to the tail end of the molecule – a process known as oligouridylation. This signals a complex of proteins known as the exosome to begin degrading the mRNA. When the exosome stalls, another chain of uridine molecules is added to restart the process. When the exosome's advance is blocked by ribosomes that have attached to the RNA to synthesize proteins, Marzluff found evidence that a complex of proteins known as Dom34/Hbs1 detaches the ribosome from the RNA, allowing the exosome to continue again. These processes are repeated until the mRNA is completely broken down.

"A postdoctoral fellow, Dr. Mike Slevin, developed a method to isolate all these molecules and sequence them. Josh Welch, a graduate student

with Dr. Jan Prins, professor of computer science developed a program that allowed us to actually map these onto the genome," said Marzluff.

The entire process takes around 45 minutes to completely degrade the histone mRNA. Using a high throughput gene sequencer, Marzluff's team was able to quantify the number of mRNA strands that had a urisine tail and compare it with those that did not have the tails, counting the molecules throughout the process to determine the rate of degradation.

While the study focused only on one type of RNA, Marzluff said he believes the same or similar process may occur for many types of RNA. The research team's next steps are to use the same techniques to measure the degradation of RNAs throughout the cells. Marzluff published the first paper on histone RNA degradation in 1987, and credits the development of new technology - low-cost high throughput DNA sequencing – that has rapidly advanced understanding of human genetics with making this study possible, allowing researchers to look at large numbers of RNA in this case

"What's actually happening with these new sequencers is that people are figuring out ways to ask different types of questions using the same technology. It's just a matter of preparing the sample to ask the machine the question you want," said Marzluff.

Provided by University of North Carolina at Chapel Hill School of Medicine