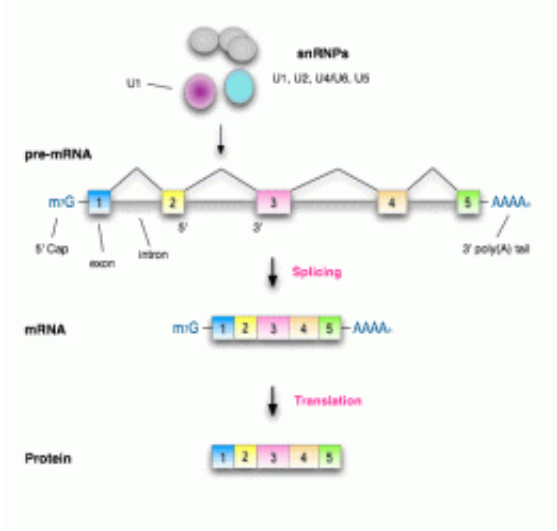
Identifying molecular guardian of cell's RNA

October 25 2010, By Karen Kreeger



From gene expression to protein. Pre-mRNA is the direct transcript of a gene, but before it is made into proteins, it needs to be processed to form messenger RNA (mRNA), including splicing of introns and joining of exons, as well as cleavage/polyadenylation at the end of the transcript. The splicing reaction is mediated by the spliceosome, usually comprised of U1, U2, U4/U5 and U6 snRNPs that come together on each intron. U1, an abundant component of the splicing machinery, binds to the 5' splice site of introns for splicing, but in addition, as the Nature study shows, it also protects pre-mRNAs from premature termination. Credit: The Dreyfuss Laboratory.

When most genes are transcribed, the nascent RNAs they produce are not quite ready to be translated into proteins - they have to be processed first. One of those processes is called splicing, a mechanism by which non-coding gene sequences are removed and the remaining protein-

coding sequences are joined together to form a final, mature messenger RNA (mRNA), which contains the recipe for making a protein.

For years, researchers have understood the roles played by the molecular machines that carry out the splicing process. But, as it turns out, one of those familiar components plays a new, and altogether unexpected role.

As senior author Gideon Dreyfuss, PhD, the Isaac Norris Professor of Biochemistry and Biophysics at the University of Pennsylvania School of Medicine and colleagues report in *Nature*, one of the splicing machinery's components called U1 has a second, equally important role in gene expression: To enable gene sequences to be read out into their RNA transcripts in their entirety, rather than have that process prematurely stopped. Dreyfuss is also a Howard Hughes Medical Institute Investigator.

The researchers revealed an unexpected function for U1 in protecting mRNA transcripts from premature termination in addition to and independent of its role in splicing.

As Dreyfuss puts it, "U1 is a guardian of the transcriptome." The transcriptome is the set of all RNA molecules in one cell.

U1 is one of a collection of RNA-protein complexes, called snRNPs, that recognize splicing junctions, excise non-coding gene sequences called introns, and join the remaining coding sequences called exons together. The Dreyfuss team previously showed that loss of SMN, a protein that helps assemble snRNPs and is deficient in individuals with the common neurodegenerative disease spinal muscular atrophy (SMA), results in altered snRNP levels and abnormal splicing.

SMN deficiency affects all snRNPs to one degree or another. The Dreyfuss team wanted to find out what would happen if just one snRNP

was missing. They started with U1.

The team's expectation was that they would detect an increase in unspliced RNA transcripts, and indeed they saw evidence of that. But, to their surprise, the majority of the genes produced a very different and striking result. Their transcripts terminated prematurely and abruptly, generally within a relatively short distance from the transcription start site of the gene.

When they sequenced the ends of the resulting truncated RNAs, they found that they had been prematurely cleaved and tagged with a long string of nucleotide building blocks called adenine. This string is a hallmark of a process called cleavage-and-polyadenylation, which normally occurs at the end of a gene's RNA transcript. The lack of U1 was causing the cleavage/polyadenylation machinery to kick into gear early.

The implication, Dreyfuss says, is that U1's normal role, in addition to splicing, is to keep the cleavage/polyadenylation machinery in check until the RNA polymerase enzyme that synthesizes the transcript reaches its finish line. The researchers propose a model in which U1 binds throughout the nascent RNA transcripts, stymieing the cleavage/polyadenylation machinery that tags along with the moving polymerase complex. This in turn protects the many potential polyadenylation signals encounter along the way, and could explain the relative abundance of U1 in cells compared to other snRNPs. The additional U1 corresponds to the greater amount needed for its additional function.

"The transcripts are under constant threat from the cleavage/polyadenylation machinery," Dreyfuss explains. "This machinery doesn't patiently wait for the transcript to reach the end of the gene; rather, the nascent RNA transcripts are subject to becoming

attacked by this machinery. It's a constant danger they face, and the U1 snRNP suppresses it."

It is, he says, "a novel role" for U1, and a critical component for correctly making mRNA. "It is essential for the integrity of the transcriptome, the landscape of all mRNA molecules in the cell," he concludes.

Provided by University of Pennsylvania School of Medicine