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Discovery Sheds New Light on Spinal Muscular Atrophy

Howard Hughes Medical Institute (HHMI) researchers have discovered a critical function for a protein involved in spinal muscular atrophy (SMA), the number one genetic killer of children under the age of two. The disease is caused when a key protein loses its ability to promote the survival and vigor of motor neurons.

According to the Families of Spinal Muscular Atrophy organization, spinal muscular atrophy affects 1 in 6,000 newborns, causing progressive muscle weakness, wasting, or atrophy as motor neurons degenerate. August is National Spinal Muscular Atrophy Awareness Month.

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— Gideon Dreyfuss

In an article published in the July 21, 2006, issue of the journal *Molecular Cell*, researchers led by Gideon Dreyfuss, a Howard Hughes Medical Institute investigator at the University of Pennsylvania School of Medicine, report that they now know the identity of a protein that is crucial for recognizing specific RNA molecules needed to process genetic information inside the cell. This process breaks down in people who have SMA.

In 1994, researchers discovered that the gene, *survival of motor neurons (Smn)*, is deleted or mutated in people with SMA. This observation strongly suggested that reduced levels of or mutations in the SMN protein cause spinal muscle atrophy.

Dreyfuss and his colleagues subsequently showed that the SMN protein is needed by all cells to produce messenger RNA (mRNA). Production of mRNA is a critical step in gene expression, and ultimately, in the production of functional proteins. Specifically, the SMN protein plays a crucial role in the genesis of mRNA from a precursor called pre-mRNA. The conversion of

pre-mRNA to mRNA takes place in the cell nucleus during a process called splicing.

Splicing requires the interaction of a large number of proteins and a group of particles called small nuclear ribonucleoproteins (snRNPs), pronounced snurps. snRNPs are critical components of the spliceosome, which snips and rearranges precursor messenger RNA molecules as they are copied from the cell's DNA. After splicing, the RNA molecules are ready to serve as templates for the cell's protein-making machinery.

The SMN complex, which is defective in people with SMA, is a large protein machine that assembles the components of the spliceosome. During assembly of snRNPs, the SMN complex must recognize specific RNA molecules called small nuclear RNAs (snRNAs) that are to be incorporated into the snRNP particles that make up the spliceosome.

In earlier studies, Dreyfuss and his colleagues had discovered a code that enables the SMN complex to distinguish snRNAs from the menagerie of other RNA molecules in the cell. However, the researchers did not know which protein in the SMN complex actually reads that code to pick out the correct RNA.

We knew the SMN complex could distinguish snRNAs from other RNAs in the cell and identify specific sequence and structural characteristics of snRNAs - what we call the 'snRNP code,' said Dreyfuss. And thus, we knew that there had to be a protein in the SMN complex that interacted directly with these snRNAs. But none of the proteins in the SMN complex had the characteristic motifs that one typically finds in RNA-binding proteins. Clearly, we were dealing with a representative of a new breed of RNA-binding protein, and the experiments described in this paper identify that protein.

In their experiments, the researchers radioactively tagged snRNAs and traced which protein in the SMN complex bound to those snRNAs. That experiment identified a protein called Gemin5 in the SMN complex as the major snRNA-binding protein. Gemin5 is one of a family of six Gemin proteins that make up the SMN complex.

Additional experiments indicated that Gemin5 in isolation binds to snRNAs and recognizes the structural features of snRNAs that Dreyfuss and his colleagues had previously identified as the snRNP code. Furthermore, when the researchers genetically reduced Gemin5 levels in cells, those cells showed reduced capacity to assemble the ribonucleoprotein components of the spliceosome.

According to Dreyfuss, the identification of Gemin5 as the snRNA-recognizing protein offers broader insight into RNA processing in the cell. This finding addresses the longstanding question of how cells distinguish one class of RNA from another, in the face of the tremendous complexity of RNAs inside each cell, he said. In this case, Gemin5 reads the signature sequence of snRNAs and confers stringent specificity on what

could otherwise be illicit RNA-protein interactions. It allows the SMN complex to be a central active orchestrator of cellular RNA metabolism, whereas it had been believed that ribonucleoprotein biogenesis occurred by a process of self-assembly.

The findings regarding Gemin5 and the function of the SMN complex are of clinical interest because reduced function of the SMN complex is the cause of SMA, said Dreyfuss. While there is currently no treatment for this devastating disorder, detailed knowledge of the SMN complex has enabled us to develop screening methods to search for small molecules that could increase the amount of SMN protein or the activity of the SMN complex in cells of patients with SMA. Such studies and a detailed understanding of how the SMN complex functions could open up important therapeutic avenues for this disease, he said.