Researchers find mechanism underlying alt. splicing of premessenger RNA into messenger RNA

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An international research team led by Tim Nilsen, Ph.D., a professor of medicine and biochemistry and the director of the School of Medicine's Center for RNA Molecular Biology, has discovered an unexpected mechanism governing alternative splicing, the process by which single genes produce different proteins in different situations. The new mechanism suggests that curing the more than half of genetic diseases that are caused by mutations in the genetic code that in turn create mistakes in alternative splicing may be considerably more complicated than biomedical researchers have previously assumed. Those diseases include a large number of cancers and many neurodegenerative diseases.

The research, titled "Dynamic regulation of alternative splicing by silencers that modulate 5' splice site competition" is published in the December 24 issue of *Cell*. Nilsen led an international team of researchers from Case Western Reserve University, Columbia University, Memorial Sloan-Kettering Cancer Institute in New York City, and the Max Planck Institute for Biophysical Chemistry in, Germany. Case post-doctoral fellow Yang Yu, Ph.D. was the lead author.

"Regular" splicing is the process by which long strings of nucleotides in a gene's pre-messenger RNA (pre-mRNA) are discarded, and the remaining strings of nucleotides are spliced together into one continuous strand of messenger RNA (mRNA) that produces one unique protein.

But regular splicing is insufficient to produce all the proteins in the human cell, where 25,000 genes surprisingly produce more than 100,000 proteins. The additional 75,000 proteins are created by alternative splicing, a process that selectively activates alternate splicing sites along the pre-messenger RNA strand to assemble different subsets of RNA nucleotides into a variety of mRNA's. Each mRNA then produces a single protein. The vast majority of genes utilize alternative splicing.

Although it creates needed proteins, alternative splicing can also lead to problems. Mistakes in alternative splicing caused by changes (mutations) in DNA sequences create more than half of all genetic diseases. For instance, sometimes the resulting mRNA includes nucleotide sequences that should have been deleted.

Because the molecular mechanisms responsible for splice site choice remain poorly understood, Nilsen and his colleagues decided to study how some potential splice sites are "silenced," preventing them from forming splices while causing other, weaker splice sites to make splices instead. To do this they created a synthetic pre-mRNA containing two alternative splice sites, a weak site and a much stronger site downstream of it. Only one of them can be activated to make each possible mRNA, and whichever one the genetic machinery chooses, it then deletes a section of pre-mRNA from that beginning point to a common ending point downstream of both beginning splice points.

The research team randomized all possible combinations of nucleotides in a sequence of 12 nucleotides just downstream of the stronger splice site and in 12 just upstream of it, their exact positions chosen so that they would be as close as possible to the splice site without interfering with the very nearest nucleotides, which are required to complete the splices. This work was done both in vitro and in vivo, with similar results.

In control synthetic pre-mRNA's where the weak upstream splice site was left out, splicing always took place between the preferable downstream splice site and the common splice ending point. But when the weak upstream site was present, changes in nucleotide type and order near the downstream splicing site sometimes silenced it and sometimes did not.

Using bioinformatics techniques, the researchers organized the 89 unique intronic silencer sequences they found into four distinct clusters, while the 47 unique exonic silencers formed two clusters. That more than 130 potential mRNA's could be prevented from being manufactured was highly unexpected. That means that small changes in a nucleotide sequence near a splice point can lead to large changes in splice site choice and proteins produced.

If one considers the metaphor that pre-mRNA is a long sentence, Nilsen says, then nucleotides and splice sites are the words of the sentence. "Adding or deleting one word," he says, "can radically change the meaning of the sentence."

According to Joseph Nadeau, Ph.D. chair of the genetics department in the School of Medicine, the most important conclusion of Nilsen's research is that "it's context, not code that's important." He says the current assumption of biologists is that there are rules that govern biological phenomena like alternative splicing, and those rules are hidden in the DNA code. So as soon as we break the code, this assumption predicts, we will be able to control alternative splicing and start curing the genetic diseases it causes.

In Nilsen's view, the context—the changes in no more than two dozen nucleotides near a possible splice point—is part of the genetic code. His team's discovery means that the code is much more complicated than scientists ever anticipated, and that fact will likely delay the day

scientists can begin to intervene in the code to cure genetic diseases.

Source: Case Western Reserve University