MedicalXpress

RNA may play larger role in cell's gene activity

June 28 2007

Large, seemingly useless pieces of RNA - a molecule originally considered only a lowly messenger for DNA - play an important role in letting cells know where they are in the body and what they are supposed to become, researchers at Stanford University School of Medicine have discovered.

The finding implies that ancient RNA molecules can orchestrate gene activity across vast portions of the human genome - a cell's genetic blueprint. It also suggests they may be important in cancer development and stem cell maintenance. Overall, the work adds another brick to the growing wall of evidence suggesting that RNA is more than a mere genomic servant.

RNA is best known for ferrying protein-coding instructions from DNA, once thought to be the master molecule of the genome, to the cell's assembly factories. But cracks in this theory began to appear when it became evident that many RNA molecules aren't capable of making protein. While more recent research has shown that small bits of RNA can silence individual genes by interfering with their expression - a la Stanford professor Andrew Fire's recent Nobel work - longer pieces, called non-coding RNAs, have been more perplexing.

"These ncRNAs have long been molecules of mystery," said John Rinn, PhD, a postdoctoral scholar in the laboratory of Howard Chang, MD, PhD, assistant professor of dermatology. "They look just like they should code for proteins, but they don't."

Although ncRNAs have been shown to affect the expression of neighboring genes, the relative abundance of the molecules - accounting for about half of the DNA transcribed in the cell - suggests that they may have a wider sphere of influence than previously thought. Now Rinn, Chang, and their collaborators have discovered that ncRNAs can influence gene expression patterns at distant locations in the cell.

"We were surprised to find that at least one of these molecules can suppress genes on a completely different chromosome," said Chang. "This opens up the whole genome to potential regulation by ncRNAs." The research will be published in the June 29 issue of the journal *Cell*.

The researchers were investigating how human skin cells, or fibroblasts, know where they are in the body. They had previously shown in different types of cells that groups of genes known as HOX act as a sort of global positioning system by maintaining unique patterns of expression over many generations of cell division. But until Rinn used a new type of gene chip called a tiling array in the new study to home in on nearby regions of DNA, they didn't know how the HOX expression patterns themselves were determined.

"I like to think of it as genomic scuba diving," said Rinn of the new experiments. The tiling array allowed him to map the boundaries of the regions around four clustered sets, or loci, of HOX genes, known as HOXA through HOXD, to near-nucleotide resolution. That's somewhat like zooming in on a single home from a satellite map on Google Earth. "It gives us an up-close, unbiased view of what's actually happening at the chromosomal level," said Rinn.

Not only did Rinn locate many previously unknown ncRNA genes nestled among the HOX genes, he also identified areas that serve as shared landing pads for proteins that either activate or suppress the neighboring regions. "It's a striking pattern," said Rinn. "Like a light

switch, the same stretch of DNA can be used to turn genes either on or off, depending on their protein partners." But then Rinn looked more deeply.

The fact that the ncRNAs have remained virtually unchanged over millions of years suggests they may be playing non-traditional but vital roles in gene expression. The researchers found that depleting one ncRNA dubbed HOTAIR, in the HOXC region on chromosome 12 of a skin cell, significantly increased the expression of HOXD genes on chromosome 2. The finding marks the first time that ncRNA has been shown to affect gene expression on a chromosome other than its own.

The researchers believe that HOTAIR functions by affecting chromosomal packing in the nucleus. Inactive chromosomal regions are tightly wound around proteins called histones and cannot be copied into RNA. Loss of HOTAIR in skin cells specifically frees the HOXD control region for binding by activating proteins.

"Next we need to find out how these RNAs work structurally," said Rinn, "and what upstream regulatory molecules might be controlling their expression." They have one clue: HOTAIR binds to and activates a group of enzymes called the Polycomb Repressive Complex 2 that modifies histones and helps them wind up the DNA.

The researchers' interest is more than just theoretical. Polycomb proteins are improperly regulated in some types of cancers. HOX gene expression patterns are likely important to keep stem cells from improperly differentiating into skin, muscle, or other tissues. Understanding how ncRNAs affect these processes will have important implications for cancer therapies and stem cell research, they believe.

"We are really interested in how ncRNA finds its putative target in the genome," said Chang. "There remains a whole level of biological

complexity to be explored, including how HOTAIR knows where to go, how it talks to other factors and how it controls histones."

The work will also provide insight into the evolution of gene regulation. Because RNA is thought to have preceded DNA in the evolutionary timeline, it makes sense that it still plays a role in controlling DNA's function.

Rinn and Chang's Stanford collaborators on the research include cancer biology graduate students Jordon Wang and Xiao Xu; surgical postdoctoral scholar Samantha Brugmann, PhD; research assistant Henry Goodnough; and Jill Helms, PhD, associate professor of surgery. Non-Stanford collaborators include graduate student Michael Kertesz and Eran Segal, PhD, at the Weizmann Institute of Science, and Sharon Squazzo and Peggy Farnham, PhD, at the University of California at Davis.

Source: Stanford University Medical Center