

MARCH 22, 2002

Researchers Discover New Mechanism that Targets and Destroys Abnormal RNA

Research teams from two Howard Hughes Medical Institute (HHMI) laboratories have identified a new mechanism that cells use to recognize and destroy abnormal messenger RNA (mRNA). It is likely that cells employ the new mechanism, called nonstop decay, to target and destroy RNA molecules that contain errors.

Although nonstop decay is a normal RNA-policing mechanism, the researchers suggest that it might interfere with some drug treatments for cystic fibrosis and other genetic diseases. The new studies suggest that nonstop decay can be thwarted, which may make drug treatments for these diseases more effective.

The discovery of nonstop decay is reported in the March 22, 2002, issue of the journal *Science* by research teams led by HHMI investigators Harry C. Dietz at The Johns Hopkins University School of Medicine, and Roy R. Parker at the University of Arizona.

Messenger RNA molecules are the genetic templates for proteins. In constructing proteins, the mRNA template is transcribed from DNA genes and transported to the ribosomes – the cell’s protein “factories” that are large complexes of protein and RNA. Given the importance of mRNA as an information-carrying molecule, the machinery that regulates mRNA levels and destroys faulty mRNA is critical in ensuring that errors in the genetic code are not passed on to proteins.

According to Dietz, his research team first believed that nonstop decay was similar to nonsense-mediated decay, the cell’s principal mechanism for destroying faulty mRNA that contains abnormal early stop signals called “nonsense” codons. Many genetic mutations or errors in transcribing mRNA result in nonsense codons that fail to code for any amino acids, the building blocks of proteins.

“At the beginning, we had the hypothesis that an mRNA *nonstop* transcript may behave very much like a *nonsense* transcript,” said Dietz. “In both circumstances the ribosome is deprived of the potential to see a bona fide termination codon in its proper context.”

One critical clue that the nonsense and nonstop mechanisms were different emerged from the work of Pamela A. Frischmeyer, in Dietz's laboratory, who showed in experiments in yeast that nonstop decay shared none of the enzymes required for nonsense-mediated decay. "We found that nonstop decay was an entirely new mRNA turnover mechanism that had none of the properties of nonsense-mediated decay, or of normal mRNA turnover in the cell," said Dietz. Additional experiments showed that the same nonstop decay mechanism found in yeast was also conserved in mammalian cells, he said.

"Once we recognized this conservation, the question arose as to why evolution would develop and maintain this mechanism," said Dietz. When the scientists searched genomic databases, they found a surprise: One percent of genes in both humans and yeast produce mRNAs containing specific sequences that would trigger degradation of the RNA by nonstop decay.

"These sequences were often conserved through evolution in a given message," said Dietz. "If the net result was simply to be wasteful, to cause degeneration of transcripts, then we would expect that they would not be conserved. But the fact that they were conserved suggests that these nonstop transcripts, and the proteins that could result from them, may have some importance in normal development."

According to Dietz, nonstop mRNA transcripts might be important in enabling production of shortened proteins that are needed at specific stages of development. At later stages of development, when these truncated proteins are no longer needed, their mRNA could easily be destroyed by nonstop decay.

Dietz and his colleagues also explored whether nonstop decay reduces the effectiveness of drugs currently being tested to treat genetic diseases in which mutations cause premature termination of protein production.

"A specific example is the testing of drugs called aminoglycosides to treat cystic fibrosis and other diseases caused by premature termination codons," said Dietz. "The idea is that these drugs would allow read-through of such codons, to generate adequate levels of full-length functional proteins. Unfortunately, these drugs have not performed very well," he said.

"Our studies of the effects of one such drug on yeast indicate that this read-through generates mRNAs that trigger the nonstop decay mechanism to degrade them," said Dietz. This finding offers the promise that drugs that inhibit nonstop decay might enable aminoglycoside drugs to function as effective treatments for some genetic diseases.

"Termination codons are present in about one-third of human disease genes, representing literally thousands of genes," said Dietz. "So this dual-drug treatment strategy could be relevant to a large number of human disorders, including cystic fibrosis and muscular dystrophy."

In the second *Science* paper, lead author Ambro van Hoof, in Parker's laboratory, did experiments that revealed the specific cellular machinery that

produces nonstop decay. According to Parker, those experiments showed that a multi-enzyme complex called the exosome is important for nonstop decay.

The exosome is a collection of enzymes called exonucleases that snip apart RNA molecules. In their experiments, van Hoof, Parker and their colleagues set out to see if the exosome was involved in nonstop mRNA decay.

“It was known that the exosome was involved in a variety of RNA degradation processes in the cell, probably controlled through specific adapters, although we really don’t understand the mechanisms well,” said Parker. According to Parker, the adapter protein, which is attached to the exosome, somehow recognizes the nonstop RNA and attaches to the ribosome.

Parker, van Hoof and their colleagues concentrated on a specific adapter protein called Ski7p, because earlier studies had shown that it had characteristics that made it a good candidate for involvement in nonstop decay. “So, our hypothesis was that Ski7p recognizes these ribosomes holding nonstop mRNAs, and recruits the exosome to degrade the defective messages,” said Parker.

The scientists’ experiments in yeast revealed that exosomes are, indeed, required for nonstop decay and that Ski7p does bind to exosomes. Their studies also showed that when Ski7p is mutated to disrupt its function, the nonstop decay machinery ceases to operate. According to Parker, additional studies are underway to understand how Ski7p recognizes and begins degradation of the nonstop mRNA buried deep within the ribosome.