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Learning How Cells Stop the Nonsense

Howard Hughes Medical Institute researchers have discovered important distinctions between two mechanisms that cells use to respond to faulty messenger RNA.

The scientists say that studying the inherent differences in the two mechanisms, nonsense-mediated mRNA decay (NMD) and nonsense-mediated altered splicing (NAS), may improve understanding of how cells guard against potentially catastrophic errors in gene expression.

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— **Melissa J. Moore**

In an article published in the October 11, 2002, issue of the journal *Science*, HHMI investigator Harry C. Dietz and colleagues at The Johns Hopkins University School of Medicine present evidence that NMD and NAS are distinctive cellular mechanisms. Previous theories held that NAS was little more than a secondary consequence of the degradation of faulty messenger RNA (mRNA) by NMD.

Nonsense mRNA contains errors that produce a premature stop signal when the cells protein-making machinery attempts to read the genetic information. Any resulting proteins would be truncated and functionally useless or even harmful. NMD is known to degrade nonsense mRNA before they produce abnormal proteins, but NAS appears to splice the new mRNA within the nucleus to excise the aberrant stop signal.

The puzzle, said Dietz, is that the cell appears to know when nonsense mRNA has been created before it even leaves the nucleus to enter the cytoplasm, which contains the ribosome, the cells protein-making factory.

For many years there has been the observation that the decay of nonsense mRNAs in mammalian cells appears to occur in the nuclear fraction of those cells, said Dietz. That was confusing because the only mechanism to interpret the coding potential of an mRNA would be the translating ribosome in the

cytoplasm. So there's been a tremendous amount of controversy regarding whether there is a functional entity equivalent to nuclear nonsense surveillance, which would challenge all of the tenets regarding how the cell interprets the quality of mRNA.

Several theories had been advanced to explain the conundrum, said Dietz, but they did not explain it satisfactorily. There was a lot of hand-waving going on with those possibilities, he said. It appeared to be a very dedicated effort to preserve what we knew — or thought we knew — about how the nucleus functions.

In their studies, Dietz and his colleagues explored how components of the nonsense mRNA-surveillance machinery might insinuate themselves in the nucleus. Specifically, the scientists sought to determine whether NAS depended on two proteins, called regulator of nonsense transcripts 1 and 2 (rent1 and rent2), that are part of the cell's nonsense mRNA surveillance machinery.

Working with cultured human cells, the scientists used a method called RNA interference (RNAi) to selectively eliminate the function of either rent1 or rent2 to see whether the loss of either protein had any effect on NMD or NAS. RNA interference involves inserting into cells double-stranded RNA that corresponds to a target mRNA. In a process still not fully understood, the double-stranded RNA interferes with the target by causing its degradation. Thus, the scientists were able to shut down the action of either rent1 or rent2, by using RNA interference to eliminate the mRNA that codes for the two proteins.

What we found was quite remarkable, said Dietz. We found that if we inhibit expression of rent1 with RNAi, that we inhibited NMD as we expected, as well as NAS. So, that documented that the nonsense surveillance machinery is involved in altered splicing of a nonsense RNA, an exclusively nuclear event. But when we targeted rent2 expression, we recognized that nonsense-mediated altered splicing was unaffected despite achieving comparable inhibition of NMD. So that tells us that NAS is not simply a secondary consequence of NMD.

In additional studies, Dietz and his colleagues inserted two different mutant forms of rent1 into the cells in which they had already blocked normal rent1 by using RNAi. Using this technique, which they call allele-specific RNA interference, Dietz's team found that one mutant inhibited both NMD and NAS, but another inhibited only NMD.

This proved that NMD and NAS are genetically separable functions of rent1, said Dietz. It constitutes proof that NMD and NAS utilize some of the same machinery, but they are not functionally identical.

Finally, the researchers explored whether rent1 might actually enter the nucleus, which would offer the possibility that nonsense RNA is detected by the surveillance machinery while still fresh from transcription.

We treated cells with a specific inhibitor of the major protein export complex that moves proteins out of the nucleus, said Dietz. We thought if we poisoned the export pathway, then we could reveal any nuclear life of rent1. And that's exactly what we saw, he said.

According to HHMI investigator Melissa J. Moore, the *Science* paper by Dietz and his colleagues — and a recent *Molecular Cell* paper on NMD and NAS by Miles Wilkinson of the University of Texas M. D. Anderson Cancer Center — provide insights into the relationship between the two processes. Moore, who is at Brandeis University, wrote in a *Perspectives* article in the journal *Science* that the two papers begin to unravel this mystery by showing that NMD and one type of NAS are functionally distinct processes that rely on different but overlapping sets of proteins.

Moore also emphasized the importance of the allele-specific RNA interference technique used by Dietz — in which the scientists inserted mutant forms of a protein into a cell in which the wild-type protein had been inhibited by RNA interference. I think that allele-specific RNA interference is going to be very useful to other researchers in studying a broad range of areas, said Moore in an interview. For example, you could study alternative splicing of mRNA by selectively knocking out one form and not the other.

According to Dietz, the latest discoveries about the roles of rent1 and rent2 in NMD and NAS represent the beginning of a long road of inquiry. Among the major unanswered questions, he said, is how the surveillance machinery influences the processing of so-called pre-mRNA — newly transcribed RNA before it has been rearranged by splicing into mRNA with a contiguous coding sequence that can be scrutinized for a premature termination codon. In addition, said Dietz, the finding could lead to better understanding of if and how the surveillance machinery affects RNAs that are not meant to be templates for proteins. I think that whatever we find is going to be new and exciting, he said.