

Researchers discover critical detail of cellular defense against genetic mistakes

Researchers are closing in on a completed diagram of how human cells protect themselves against constant genetic mistakes that contribute to most diseases, according to a study to be published in the April 18 edition of the journal *Cell*.

The blueprint for the human body is encoded in genes. Gene expression is the process by which those blueprints are converted into proteins that make up the body's structures and send its signals. When molecular biologists began analyzing the complete set of human genes (the human genome) in 2001, one surprise was that humans have as few as 30,000 genes when, given their complexity, they should have more than 100,000. How can humans have one-fifth as much genetic material as wheat, for instance, or share one quarter of their genes with fish?

One answer is that humans do more with fewer genes. While genes consist of chains of deoxyribonucleic acids (DNA), they are put into practice by chains of ribonucleic acid chains (RNA), which are modified copies of DNA. Messenger RNA (mRNA) is transported to cellular factories called ribosomes that receive instructions for building proteins by "reading" mRNA templates, a process called translation. Remarkably, about 75 percent of human genes code for more than one protein through a process called alternate RNA splicing. Unfortunately, the more intricate the splicing process, the greater the opportunity for error. More than one-third of alternatively spliced mRNAs are flawed, and must be destroyed before they can cause harm. Thus, cellular processes that detect and eliminate processing errors are vitally important to effective gene expression.

In recent years, researchers at the University of Rochester Medical Center have revealed the existence of a natural surveillance system called nonsense-mediated mRNA decay (NMD) that determines which mRNAs are fit to serve as protein templates and sees to the destruction of those with flaws. Researchers hope to tweak the process such that it catches more genetic errors in some cases, or leaves more templates for helpful proteins in place in others, based on the disease at hand. To do so will require a highly detailed knowledge of the NMD pathway.

"The current results uncover a critical and previously unappreciated step during the natural process that finds flaws in mRNAs," said Lynne E. Maquat, Ph.D., J. Lowell Orbison Endowed Chair and professor of Biochemistry & Biophysics at University of Rochester Medical Center, director of the University of Rochester Center for RNA Biology and lead author of the *Cell* piece. "This work has important implications for our understanding of how one of the human cell's most important activities, protein synthesis, undergoes quality control."

An Elegant Process Emerges

Over time, genes evolve to show changes in their makeup. Some changes, or mutations, have no impact, some provide advantages making organisms more likely to survive, and others cause disease. One frequently occurring, damaging class of mutation is the inclusion of premature "stop reading" signals (stop codons) within mRNAs. Called "nonsense" mutations, they order the process to stop reading part way through the genetic instructions. Such mutations result in the building of incomplete, disabled proteins that sabotage natural processes by competing for spots usually held by their full-length counterparts, or by simply not working. Mutations of this type cause genetic syndromes and contribute to many diseases, including cancer. Since truncated proteins are potentially hazardous, the NMD pathway has evolved to

eliminate the mRNAs that encode them.

From studying genetic diseases, Maquat theorized seven years ago that there must be two types of translation, the process by which instructions encoded in mRNAs are read during protein building. An early “pioneer” round checks all newly built mRNAs for errors, and initiates NMD when errors are detected. Subsequent “steady-state” rounds then direct the mass production of normal proteins based on “NMD-approved” mRNAs. Over time, the Maquat lab, along with other labs, has identified a number of protein complexes that form during the intricate process by which cells analyze each mRNA for flaws.

In the past, her team showed, for instance, which proteins bind to each end of mRNA during the pioneer and subsequent steady-state rounds of translation, and how the pioneer round, cap-binding protein promotes the recognition and decay of flawed mRNAs. The team also demonstrated how other complexes that identify flawed mRNAs form near exon-exon junctions, the places where each “must read” section of the mature mRNA template is joined to the next by RNA splicing.

Past work by Maquat’s team further revealed that much of the NMD quality review depends on the physical spacing of proteins bound to the mRNA chain. If a stop reading signal occurs too far ahead of the final exon in the chain, as marked by an exon-exon junction complex (EJC), the cell concludes that the stop codon has mistakenly fallen in the middle of a set of instructions. These mRNAs are degraded. They also found that the EJC contains human up-frameshift (UPF) proteins that play a role in NMD.

In their latest search for detail, Maquat and colleagues determined that the delivery of a given faulty mRNA to the degradation machinery requires first the active shutdown (translational repression) of protein building based on that mRNA. In the study’s key finding, experiments revealed that repression of protein synthesis during NMD is controlled the attachment of phosphate groups to human UPF1, researchers said. Human cells have evolved such that phosphorylation, the attachment of phosphate groups to proteins, is used in many scenarios like a switch to turn processes on or off.

Based on their findings, Maquat and colleagues propose the following new model for NMD: When a nonsense stop codon is detected, UPF1 together with the enzyme that directs its phosphorylation interacts with the EJC. The same step makes possible the attachment of phosphate groups to UPF1. Once phosphorylated, UPF1 interacts directly with and inhibits the function of eukaryotic initiation factor 3 (eIF3), which would otherwise direct protein building based on that mRNA sequence.

Normally, eIF3 drives a key change in a complex (40S/Met-tRNAⁱMet/mRNA) that consists of mRNA and part of a functional ribosome. The binding of phosphorylated UPF1 to eIF3 prevents this complex from going on to form a complex (80S/Met-tRNAⁱMet/mRNA) that is capable of driving translation and consists of mRNA and the completed functional ribosome.

The team corroborated the importance of eIF3 as a target for translational repression during NMD using an experiment with an mRNA sequence from cricket paralysis virus. Where human cells use eIF3 to initiate translation, the cricket virus mRNA sequence does not. Researchers found that the non-eIF3 translation initiation directed by the cricket virus sequence in mammalian cells was resistant to NMD, and thus that eIF3 is a must for the translational repression that makes NMD possible.

In Maquat’s model of NMD, phospho-UPF1 not only inhibits the pioneer round of translation so that the translational machinery “falls away” from the flawed mRNA at hand, but also recruits degradative enzymes to that mRNA.

Along with Maquat, the study was authored by post-doctoral associates Olaf Isken, Yoon Ki Kim and Nao Hosoda under the auspices of the Medical Center. Greg L. Mayeur and John W.B. Hershey from the

Department of Biological Chemistry at the University of California at Davis provided important reagents and advice. This work was supported by the National Institutes of Health.

“Our study provides the first evidence that translational repression does indeed occur during NMD in mammalian cells,” Maquat said. “One implication of these results is that we have a new target by which the decay of faulty mRNA can be prevented. In cases where a nonsense codon occurs in a gene supplying an essential protein, and thus causes disease via protein shortage, we may be able to design drugs that suppress related decay. That could restore the supply of an mRNA that can direct the cell to synthesize full-length, functional protein.”

Source: University of Rochester

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