New method prevents premature halt in protein synthesis in certain genetic illnesses

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Improperly formed proteins can cause a host of serious illnesses, from muscular dystrophy to cystic fibrosis. A question of enormous import in research, beyond the challenge of determining how malformed proteins contribute to specific disease processes, is figuring out ways to prevent or reduce the pathologies they cause.

Today, a team of researchers at Cold Spring Harbor Laboratory (CSHL) describes in *Nature Biotechnology* their success in paradoxically inhibiting a process cells have evolved to prevent imperfect proteins from being synthesized in the first place. Sometimes, it turns out, it's beneficial to allow protein synthesis to continue—but only in very particular cases.

A natural process called nonsense-mediated decay, or NMD, provides cells with the ability to detect errors in the coded RNA messages, called transcripts, that are copied from DNA when genes are activated. The copying process is called transcription. Activation of a gene induces a cell to make an RNA copy of its code, edit unneeded segments out of that message, and splice together a final version of the message that provides cellular factories (ribosomes) with a template to make one specific protein.

Mutations in genes can foul up the works, right from the start. Although mutations come in many varieties, one type, called nonsense mutations, involve the seemingly innocuous change of a single letter in the coded message—a change that causes the gene's message to prematurely read

"stop."

When things are working properly, the stop signal comes at the very end of the coded message. This tells the ribosome to end the protein manufacturing process at the appropriate place.

When the stop signal comes too soon, due to a faulty letter in the code, the process ends prematurely and various outcomes are possible. Often, the error is noted by the cell and the NMD process is engaged, as a way of preventing bad outcomes. Via NMD, the faulty message is degraded and little or no protein is made. Other times, an abnormally short version of the protein is still manufactured. Such truncated proteins are sometimes harmless. Other times they are partially useful to the cell. Still other times a too-short protein can have toxic effects.

A team led by CSHL Professor Adrian Krainer, an authority on the complex processes cells use to splice together the RNA messages (called mRNAs) that serve as protein templates, has now devised a method to prevent NMD from being engaged. The critical caveat is: his team has determined how to deactivate this cellular quality-control mechanism only when it is advantageous - only when it will result in the therapeutic restoration of protein manufacture, whether the resulting protein is full-length or at least partly functional.

Part of the challenge is knowing in what situations it's going to be advantageous to inhibit NMD. The details are technical, involving how far - how many coded RNA letters - a premature stop signal occurs relative to a location on the message that serves as a loading site for an EJC (exon junction complex). EJCs are like chapter marks or tags deposited in the cell's nucleus as a gene's copied RNA message is edited, or spliced.

The innovation tested successfully by Krainer and his team, including

first author of the new paper, Tomoki Nomakuchi, along with Isabel Aznarez and Frank Rigo, involves the use of a molecule called an ASO (antisense oligonucleotide) that will block the site normally occupied by a particular EJC.

"We only want to dislodge or replace the EJC at one spot in the gene's spliced RNA message," explains Krainer. "We design and test an ASO that will prevent a particular EJC from binding to the message; the others are unaffected."

Why? Because when the cell detects an EJC within a certain distance from a "stop" signal in a gene message, that is when it calls the NMD machinery into action, to halt decoding of the message and resulting in its degradation.

The trick is do this only in gene messages in which a premature stop signal, if ignored, will nevertheless result in the production of a full-length, or at least partly functional protein - one that will have a helpful impact in a disease process.

"We are interfering with the NMD machinery's ability to target such mRNAs for destruction," Krainer says. "This means the cell will make more protein. It may still be truncated - but that can be beneficial. In other cases, a truncated protein would not be functional, or could even be toxic." In the latter cases, either it would not make sense to intervene with an ASO; or, an ASO could be combined, Krainer says, with so-called read-through drugs. These are drugs - still experimental - that prevent premature stop signals from halting protein production by ribosomes, although NMD tends to limit their efficacy. The current work shows that combining a read-through drug with an appropriate EJC-blocking ASO results in cells manufacturing substantially more full-length protein.

More information: "Antisense-oligonucleotide-directed inhibition of nonsense-mediated mRNA decay" appears December 14, 2015 in *Nature Biotechnology*. DOI: 10.1038/nbt.3427

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