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Protein synthesis: stop the nonsense

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Abstract

The amino acid sequence of proteins is specified by trinucleotides (codons), and their ends by one of three stop codons. A new study reveals how a subtle biochemical alteration can reprogram stop codons into amino acid-encoding codons at the messenger RNA (mRNA) level, without altering the DNA from which the mRNA is transcribed.

Nonsense suppression occurs when a stop (or nonsense) codon of mRNA (UAA, UAG or UGA) is decoded by the translation machinery as an amino acid, rather than eliciting termination of protein synthesis. Decoding of mRNA takes place in the ribosome, where each mRNA codon is matched with the anticodon of an aminoacyl-tRNA. The latter is a tRNA (transfer RNA) that has been charged with the amino acid corresponding to its anticodon by its cognate aminoacyl-tRNA synthetase (aaRS). Normally, stop codons in mRNA are recognized by proteins called release factors (RF1 and RF2 in bacteria, eRF1 in eukarya); none of the standard tRNAs have anticodons complementary to stop codons. One can imagine that if a tRNA were to suffer a mutation in its anticodon such that it became complementary to a stop codon (and this mutant tRNA were otherwise recognized normally by its aaRS and the rest of the translation machinery) it might lead the ribosome to misinterpret a stop codon. Indeed, nonsense suppression by mutated (“suppressor”) tRNAs is well documented. On page XXXX of the issue, Karijovich and Yu¹ report the unexpected discovery of nonsense suppression through post-transcriptional isomerization of the uridine (U) of stop codons into pseudouridine (Ψ) in the mRNA, rather than through an aberrant tRNA. This finding raises fundamental questions about the biochemistry of translation and suggests a novel way of treating many genetic diseases.

The experimental results of Karijovich and Yu¹ are surprising because of the mechanism by which release factors are thought to recognize stop codons, and because of the structural similarity between Ψ and U. Crystallographic structure determination of the bacterial ribosome with release factors caught in the act of recognizing stop codons^{2,3} indicates that recognition of the 5' U of all three stop codons by RF1 and RF2 is through hydrogen bonding between protein backbone functional groups and the Watson-Crick face of that nucleotide (Figure 1A, B). Although Ψ and U differ in that the former has a carbon-carbon glycosidic bond and also a imino group in the major groove, the Watson-Crick faces of these two pyrimidines are identical (Figure 1C). Thus, release factors should be insensitive to conversion of stop codons to ΨAA, ΨAG, or ΨGA. Some property of Ψ other than its ability to Watson-Crick base-pair must give rise to nonsense suppression. Ψ binds water through its major groove imine, and this makes Ψ-containing RNAs stiffer⁴. It could be the increased energy needed to desolvate the Ψ-containing mRNA, or the greater difficulty in unstacking the isomerized stop codon from the previous codon that results in nonsense suppression. Regardless of its physical-chemical basis, the new results point to the importance of factors other than Watson-Crick base pairing in stop codon recognition.

Karijovich and Yu¹ demonstrate nonsense suppression through termination codon pseudouridylation both *in vitro* and *in vivo* in yeast. When they characterized the proteins

synthesized by nonsense suppression, these authors uncovered another surprise. Rather than incorporating a random amino acid at the site occupied by the isomerized stop codon, the translation machinery incorporates serine or threonine at Ψ AA and Ψ AG and tyrosine or phenylalanine at Ψ GA. This is noteworthy because while the two sets of amino acids have chemical commonalities (threonine and serine both have a hydroxyl group, and tyrosine and phenylalanine share a phenyl ring), the anticodons of tRNAs for these four amino acids do not have any obvious complementarity to the stop codons. Mechanistically, this implies that pseudouridylation of stop codons leads not only to loss of recognition by release factors, but to a gain of recognition by specific aminoacyl-tRNAs. The fidelity of normal translation is enhanced through a proofreading process in which the accuracy of codon-anticodon pairing is communicated across the ribosome to the aminoacylated (acceptor) end of tRNA. Perhaps pseudouridylation of stop codons also affects this process.

Ψ is produced from U residues of cellular RNAs by site-specific enzymes called Ψ synthases⁵. Eukaryotes and archaea have a versatile class of Ψ synthases called the H/ACA ribonucleoprotein (RNP)⁶. These all share four proteins, and differ in having a different RNA (with conserved “H” and “ACA” sequence elements). The RNA component of the RNP is called a “guide RNA” because it has a stretch of nucleotides complementary to the sequences that flank the uridine of the substrate RNA targeted for pseudouridylation. This sequence complementarity is necessary and sufficient for directing the H/ACA RNP to pseudouridylate a cellular RNA *in vivo*. Indeed, Karijovich and Yu¹ employ a custom-designed H/ACA guide RNA to target stop codons for pseudouridylation in their yeast experiments, and these authors point out that this would be an attractive approach to treating genetic disorders that result from premature termination of translation, since all that would be required would be delivery of a H/ACA guide RNA that pseudouridylates the defective mRNA, rather than having to correct the mutation at the level of DNA. It is noteworthy that upwards to a third of genetic disorders and many cancers are due to mutations that introduce premature stop codons⁷. More broadly, it is possible that nature is already employing this kind of “gene therapy” to increase the coding capacity of genomes. Karijovich and Yu¹ have found several candidate mRNAs whose stop codons could be subject to pseudouridylation by previously described H/ACA guide RNAs. Such mRNAs would produce a shorter protein in their unmodified state, and a longer protein (that ends at a second, unmodified stop codon) when the first stop codon is pseudouridylated.

Acknowledgments

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Figure 1. Ψ synthases isomerize U into Ψ. Note the identical Watson-Crick faces of the two pyrimidine nucleotides. Thick line denotes the glycosidic bond.