Nature Reviews Genetics | Published online 25 Jul 2016; corrected 5 Aug 2016; doi:10.1038/nrg.2016.100

EVOLUTIONARY GENETICS

Knowing when to stop

> functional flexibility through context-dependent decoding of ciliate codons may represent a state of evolutionary flux

PIXTAL

The genetic code — the rules by which codons are translated into amino acid sequences — is almost universally conserved across all domains of life, although a minority of species are now known to use variant genetic codes. A new study of diverse eukaryotes has identified the first examples of species that have recoded all of their stop codons and instead rely on positional context within a transcript to mediate translational termination.

To investigate the genetic codes used by a broad range of eukaryotes, Swart *et al*. mined transcriptome data from 289 species in the Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP). In the absence of proteomics data — which would provide direct evidence of peptides that are translated using alternative genetic codes — the authors inferred variant genetic codes based on bioinformatic analysis of transcriptomic data using the previously developed FACIL tool (Fast and Accurate Genetic Code Inference and Logo). This tool uses cross-species protein alignments to predict the most likely genetic code for each species; for example, if a species consistently uses a canonical stop codon internally in a protein-coding sequence alignment, it is probable that the codon can be amino-acid-encoding in that particular species.

The vast majority of the species used the standard genetic code, but different genetic code variants were found among the 24 ciliate species analysed, adding to previous examples of genetic code flexibility in this group of species. Most notably, the ciliates *Condylostoma magnum* and an unclassified species of the *Parduzcia* genus are the first examples for which all three stop codons (UAA, UAG and UGA) are predicted to have been reassigned to encode amino acids (glutamine or tryptophan), such that all 64 possible codons are amino-acid-encoding.

Evidence that these canonical stop codons indeed encode amino acids was provided by several means. The presence of these codons internally in the coding regions of single-copy essential genes, combined with amino-acid-based evolutionary constraint both upstream and downstream of these codons, indicated that these 'stop' codons lead to amino-acid incorporation and continued translation, rather than translational termination. In addition, proteomics and ribosome profiling confirmed continuous productive translation through these codons. Although glutamine is probably incorporated at UAA and UAG codons in *C. magnum* using cognate tRNAs encoded by the macronuclear genome (similarly to other ciliates), it is still unclear which tRNA recognizes its UGA codons. For these codons either a cognate tRNA encoded by the mitochondrial genome or a near-cognate tRNA encoded by the macronuclear genome might be used.

So, if all codons are amino-acid-encoding, how does translational termination occur at the intended sites in these species? The investigators found that canonical stop codons are still used for translational termination at the carboxyl termini of proteins, implying that stop codons are ambiguous in these species and must be differentially interpreted by the translation machinery based on context. There was no evidence that immediately flanking nucleotides define whether a stop codon terminates translation. Instead the authors noted that ciliates have strikingly short 3′ untranslated regions (only ~21–23 nucleotides between a genuine stop codon and the polyadenylation (poly(A)) sequences, compared with >100 nucleotides in most other species). This indicates that the same codons are interpreted differently based on positional context in the transcript: the authors propose that ribosomal interactions with poly(A)-binding proteins promotes translational termination when the stop codons are close to the end of the coding regions, whereas the same codons further upstream result in continuous translation.

Such functional flexibility through context-dependent decoding of ciliate codons may represent a state of evolutionary flux and might explain why such diversity in genetic codes has been discovered among ciliate species. Given that genomic data are rapidly accumulating for a wide range of species globally, perhaps we are only at the tip of the iceberg in the search for biology's rule-breakers that have deviated from the previously assumed frozen and fixed nature of the genetic code.

Darren J. Burgess

ORIGINAL ARTICLE Swart, E. C. *et al*. Genetic codes with no dedicated stop codon: context-dependent translation termination. *Cell* http://dx.doi.org/10.1016/j.cell.2016.06.020 (2016)
FURTHER READING Baranov, P. V., Atkins, J. F. & Yordanova, M. M. Augmented genetic decoding: global, local and temporal alterations of decoding processes and codon meaning. *Nat. Rev. Genet.* **16**, 517–529 (2015)

CORRECTION

This Research Highlight has been updated from the original version published online. The final part of the fourth paragraph has been modified to correct the likely source (mitochondrial genome versus macronuclear genome) of the *Condylostoma magnum* tRNAs mediating amino-acid incorporation at the UAA, UAG and UGA stop codons. The editor apologizes for this error.