J Mol Evol (1990) 31:351–352

Journal of Molecular Evolution

Random Walking

Stop the Nonsense

The trivial names of the three stop codons have become enshrined, without explanation, in many voluminous and authoritative textbooks of molecular biology and biochemistry. They are various shades of yellow: (forever) amber, ochre, and opal. Amber was originally ambergris, an accumulation in the intestines of the sperm whale; later it became the name for a fossil resin that looked like ambergris. Ochre is a yellow iron pigment, and opal is a colored quartz. What are they doing in the table of the genetic code?

The mythology that has come down to me is that amber comes from Cal Tech. Around 1963, several graduate students were planning an evening of isolating phage mutants. One of the group, name of Bernstein, told them he wanted to take in a movie instead. His friends told him that if he would help them in the lab instead of going to the movies, they would name a mutant for his mother. A bargain was struck. The mutant turned out to be a suppressor (later found to be specifically a UAG stop codon suppressor), and it was duly named the Amber (Bernstein) mutant (Edgar 1966). I have no information on who named ochre and opal, but I presume they became hitched onto amber, something like Southern, Western, and Northern.

The three pigmented suppressors are called nonsense suppressors on the basis that a stop codon, because it was untranslatable as an amino acid, was a nonsense codon, even though the termination of polypeptide synthesis by interaction between a stop codon and a release factor is anything but nonsensical. Why not call them UAG, UAA, and UGA suppressors, and stop, rather than nonsense, codons?

The term "nonsense" dates back to Crick et al. (1957), who said "We shall assume that there are certain sequences of three nucleotides with which an amino acid can be associated, and others for which this is not possible. Using the metaphors of coding, we say that some of the 64 triplets make sense, and others make nonsense."

The term nonsense mutations was a springboard to the introduction of an even worse name: in this case an etymological monstrosity—*missense* mutations. The word missense is internally contradictory and unnecessary. It is not found in dictionaries; the closest approximation is the word *mis-sent* (as in the case of an erroneously addressed postcard), which does not relate to it. But *missense* has been complacently accepted by authors of textbooks, even eminent ones. A missense mutation is an amino acid replacement caused by a nucleotide substitution in the gene for a protein, and should be termed a replacement mutation. Such mutations may or may not be deleterious. There is no such thing as a missense codon, of course, because all 61 codons for amino acids make sense. A codon may become unassigned and therefore untranslatable if its anticodon does not exist, as might occur by deletion of the gene for the cognate tRNA. A case is CGG. This codon and its anticodon CCG could not be found in *Mycoplasma* (Andachi et al. 1989).

Remarkable Codons

The two most eminent codons are, of course, UGA and AUA, and of the two, UGA is more outstanding. I shall devote most of this space to AUA, but, first, a few words about UGA.

UGA is the only codon with three different meanings: stop, tryptophan, and selenocysteine (SeCys). Moreover, UGA can code for both SeCys and stop in the same organism! UGA is translated (in mRNAs for proteins containing SeCys) by a special tRNA, anticodon UCA, that is aminoacylated with serine, and the seryl-tRNA is then converted into SeCys-tRNA by the catalytic action of two proteins. The SeCys UGA codon in mRNA is immediately upstream from a hairpin structure that evidently prevents UGA from functioning as a stop codon in this special location (Zinoni et al. 1990). UAA is probably the evolutionary parent of UGA, assuming that codon UGA lost Trp to UGG, when anticodon UCA mutated to CCA and UAA stop codons mutated to UGA.

UGA and AUA came into prominence in 1979 when it was discovered that they were used for Trp and Met in mitochondria (Barrell et al. 1979) instead of for stop and Ile. This shattered the myth of the "frozen" universal genetic code.

AUA has for many years been scrutinized by Susumu Nishimura and others because *E. coli* had a special and unusual tRNA Ile minor for translation of AUA (Harada and Nishimura 1974). Ile minor, present at <5% of the level of tRNA Ile major, contained a modified anticodon nucleotide with an unknown chemical structure. Similar Ile tRNA species, specific for codon AUA, were found in bacteriophage T4 (Guthrie and McClain 1973, 1979), in spinach chloroplasts (Francis and Dudock 1982), and in *Halobacterium volcanii* (Gupta 1984). The finding with chloroplasts could imply that this tRNA was present in their ancestral cyanobacteria.

In 1988, enough of the unknown nucleoside in the first anticodon position was collected to enable its chemical structure to be determined by proton NMR and mass spectra followed by chemical synthesis (Muramatsu et al. 1988a). The nucleoside was determined to be cytidine with lysine attached as a side chain. The oxygen atom in position 2 of cytosine was replaced by the epsilon nitrogen atom of lysine. The new modified nucleotide was named "Lysidine." Lysidine as *C in *CAU paired with A in the third position in AUA. Unmodified CAU pairs with AUG, the universal codon for methionine.

The remarkable finding was then made that removal of the lysine side chain changed the specificity of the tRNA from isoleucine-accepting to methionine-accepting! The charging specificity of the tRNA therefore depended solely on the nucleoside in the first anticodon position (Muramatsu et al. 1988b).

What could be the effect of these findings in the evolution of the genetic code? Evidently lysidine in *CAU is a very old nucleotide because of its presence in chloroplasts. Also, Weber et al. (1990) have found post-transcriptional modifications of CAU (probably to lysidine in *CAU) in potato mitochondria. This implies that lysidine was probably used by the bacterial ancestor of mitochondria in coding for isoleucine. The mature mitochondrial tRNA had isoleucine-accepting activity but no methionine-accepting activity.

AUA codes for isoleucine in mold mt but for methionine in yeast mt. We have suggested (Osawa et al. 1989) that the switch resulted from the loss of *CAU in yeast mt, followed by structural changes in tRNA Met that enabled pairing with both AUA and AUG.

Here, perhaps, is the explanation of the change in the genetic code for AUA observed by Barrell et al. in 1979.

References

Andachi Y, Yamao F, Muto A, Osawa S (1989) Codon recognition patterns as deduced from sequences of the complete set of transfer RNA species in *Mycoplasma capricolum*. Resemblance to mitochondria. J Mol Biol 209:37–54

Barrell G, Bankier AT, Drouin J (1979) A different genetic code in human mitochondria. Nature 282:189–194

Crick FHC, Griffith JS, Orgel LE (1957) Codes without commas. Proc Nat Acad Sci USA 43:416–421

Edgar RS (1966) Conditional lethals. In: Cairns J, Stent GS, Watson JD (eds) Phage and the origins of molecular biology (essays). Cold Spring Harbor, NY, pp 166–170

Francis MA, Dudock BS (1982) Nucleotide sequence of a spinach chloroplast isoleucine tRNA. J Biol Chem 257:11195–11198

Gupta R (1984) *Halobacterium volcanii* tRNAs. J Biol Chem 259:9461–9471

Guthrie C, McClain WH (1973) Conditionally lethal mutants of a bacteriophage defective in production of a transfer RNA. J Mol Biol 81:137–155

Guthrie C, McClain WH (1979) Rare transfer RNA essential for phage growth. Biochemistry 18:3786–3795

Harada F, Nishimura S (1974) Purification and characterization of AUA specific isoleucine transfer RNA from *Escherichia coli*. Biochemistry 13:300–307

Muramatsu T, Yokoyama S, Horie N, Matsuda A, Ueda T, Yamaizumi Z, Kuchino Y, Nishimura S, Miyazawa T (1988a) A novel lysine-substituted nucleoside in the first position of the anticodon of minor isoleucine tRNA from *Escherichia coli*. J Biol Chem 263:9261–9267

Muramatsu T, Nishikawa K, Nemoto F, Kuchino Y, Nishimura S, Miyazawa T, Yokoyama S (1988b) Codon and amino-acid specificities of a transfer RNA are both converted by a single post-transcriptional modification. Nature 336:179–181

Osawa S, Ohama T, Jukes TH, Watanabe K, Yokoyama S (1989) Evolution of the mitochondrial genetic code II. Reassignment of codon AUA from isoleucine to methionine. J Mol Evol 29:373–380

Weber F, Dietrich A, Weil J-H, Marechal-Drouard L (1990) A potato mitochondrial isoleucine tRNA is coded for by a mitochondrial gene possessing amethionine anticodon. Nucleic Acids Res 18:5027–5030

Zinoni F, Heider J, Böck A (1990) Features of the formate dehydrogenase mRNA necessary for decoding of the UGA codon as selenocysteine. Proc Natl Acad Sci USA 87:4660–4664

Thomas H. Jukes
September 27, 1990