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Point Counter Point

UAR Codons for Glutamine

P.J. Keeling and W.F. Doolittle (1996) reported that UAR (TAR) codons incorporate glutamine in Hexamitidae. They state that ''the particular variation of the genetic code observed here has previously been observed only in very AT-rich nuclear genomes, where it is thought to have been favored by the same directional mutation pressure that biased the genome's composition (Osawa and Jukes 1989).''

But in our 1989 article, we said, ''A possibility would be that in ciliated protozoans, ERF (eukaryotic releasing factor) evolved to become specific for UGA so that UGA became the sole chain termination codon and UAA and UAG were removed from the terminator sites as a result of this event *rather than by AT or GC pressure*'' (emphasis added). Our proposal for UAR-bearing codons for glutamine was (and is) as follows (Osawa and Jukes 1989; Jukes et al. 1991; Osawa et al. 1992):

1. UGA becomes the sole termination codon for ciliates, and has a specific ERF.
2. UAA and UAG become untranslatable nonsense codons.
3. Anticodon UUG (UmUG) for Gln duplicates.
4. One duplicate mutates to UmUA, pairing with UAA and UAG so that these become translated as Gln (and are no longer untranslatable).
5. A duplicate of UmUA becomes CUA by a transition mutation. This provides a second anticodon for UAG.
6. AT pressure may subsequently contribute to the use of UAR for Gln by converting some CAR codons to UAR.

We recapitulated this proposal in Osawa et al. (1992). The scheme proposed by Keeling and Doolittle (1996) does not differ from ours.

References

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Response

Jukes and Osawa have expressed two main objections to our discussion of the evolution of the genetic code: that we misrepresent their model by implying that AT-pressure is required for TAR loss, and that our model is no different from that which they proposed many years ago, and maintain today.

Answering the first criticism rests in the distinction between AT-pressure involved in codon *loss* and codon *reappearance.* We did not assert that Jukes and Osawa believe that AT-pressure may drive TAR *loss,* but rather that the high AT content of ciliate and *Acetabularia* genomes ''led to the suggestion [by Jukes and Osawa] that the AT mutation-pressure that biased the overall composition of these genomes has also driven the conversion of many CAR codons to TAR'' (from Keeling and Doolittle 1996). In other words, AT mutation-pressure is evoked by Jukes and Osawa to explain the *reappearance* of TAR as glutamine codons. Indeed, this claim is made repeat-

edly in the literature (Osawa and Jukes 1989; Osawa et al. 1992), and in the present debate itself (step 6).

This distinction is relevant because the coding regions of the diplomonad species where we discovered this code are not AT-rich. This led us to propose that AT-pressure is not actually essential for codon *reappearance* either; we argue instead that any mutation that is sufficiently frequent (in this case the single transition between CAR and TAR) can fix a variant code (Keeling and Doolittle 1996). In this respect, then, our discussion does differ from their model, which explicitly evokes AT-pressure.

Moreover, our discussion also disputes the recapture of both TAA and TAG codons by a single tRNA duplication event (steps 3 and 4 of their letter). We suggest that the capture of the two codons may be fixed in the genome semi-independently based on the observation that *Tetrahymena thermophila*, *Hexamita inflata*, and *Hexamita* 50330 all contain both $tRNA^{Gln}UUA$ and $tRNA^{Gln}CUA$ (Hanyu et al. 1986; Keeling and Doolittle 1996). Since unmodified U can wobble with all four bases, in codon pairs such as CAA and CAG, the first position U must be modified to restrict mismatching. In practically every case known, this modification leaves the first position U specific for A, necessitating a second tRNA to decode NNG codons (Björk 1995). Jukes and Osawa argue that UUA could recognize both UAA and UAG if the first position U was modified to Um (Um will specifically recognize A and G). This is a distinct possibility, but Um is a rare modification, and if the original capturing $tRNA^{Gln}UUA$ was sufficient, then why do we also find $tRNA^{Gln}CUA$ in every one of these organisms that has been examined?

Lastly, although we agree that the nature of eukaryotic release factors is one key to the origin of this particular genetic code, we did not advocate the loss of release factor activity prior to the loss of TAR codons (step 1 of letter). This is a difficult point of codon-capture to explain (and has been avoided altogether by other models such as that of Schultz and Yarus 1994). The model outlined by Jukes and Osawa here and elsewhere would lead to a potentially awkward intermediate stage where some genes end in TAR, but neither TAR-recognizing tRNAs nor TAR-recognizing release factors exist in the cell. The outcome of this state in eukaryotes is not known, but in eubacteria the cognate tRNA of the penultimate codon remains covalently attached to the carboxy-terminus of the protein (Oba et al. 1991). Our suggestion is simply that TAR termination codons are drastically reduced in number, or even lost, and that this allows the loss of release factor recognition without deleterious effect. Why these codons would become reduced in number is not obvious, but the frequency of termination codons in a genome can fluctuate a great deal in the same fashion as sense codons (see the TransTerm data base: Dalphin et al. 1996), suggesting that it may be possible.

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