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Series

A cross-eyed geneticist's view V. How Sydney Brenner, Leslie Barnett, Eugene Katz, and Francis Crick inferred that UGA is a nonsense codon

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1. Introduction: the unique genetics of T4 rII

UGA was the last of the 64 codons of the genetic code to be assigned. Until then, 61 codons were shown to code for one or another of the 20 amino acids. Two codons, UAG (*amber*) and UAA (*ochre*), were established to not code for any amino acid, and hence were designated as 'nonsense' codons. Only UGA remained unallocated. To show that UGA also is a nonsense codon, Brenner *et al.* (1967) used three 'genetics' resources: (1) The chemical mutagens 2-aminopurine (2-AP) and hydroxylamine (HA). The former induces G-C to A-T and A-T to G-C transition mutations, whereas the latter induces only G-C to A-T transitions. (2) The *amber*- and *ochre*-suppressor strains of *E. coli* K12 that contain suppressor tRNAs that insert an 'acceptable' amino acid at, respectively, UAG and UAA codons in the mRNA, and thus suppress the phenotypes of mutations to these codons, and (3) the vast collection of bacteriophage T4 rIIA and rIIB gene mutants amassed by Seymour Benzer and colleagues. For an excellent introduction to the T4 rII system, see Jayaraman (2008). Garen (2002) has drawn attention to other important papers that contributed to nonsense triplet discovery.

Plaques from T4 rII mutant phage grow more rapidly on *E. coli* B lawns than those from the wild type phage (r^+). On the other hand, mutant phages do not form plaques on *E. coli* K12 whereas the r^+ phage can. This difference is the basis of T4 rII genetics. Complementation of two rII mutants is indicated if plaques form when they co-infect K12, and more than 2000 rII mutants were placed into the complementation groups rIIA and rIIB. Cross-over between two rII mutants is determined by co-infecting them on B, and then estimating the frequency of r^+ recombinants produced by plating the progeny phage on K12, where it is possible to detect one r^+ recombinant plaque among 10^8 *r*II phage plated. This system could resolve cross-over between mutant sites in neighboring base pairs (see below), and the smallest recombination frequency measured was $\sim$0.02%. Another useful property of the T4 rII system was that the first part of the rIIB gene is not critical for function and it is replaceable by varying lengths of the rIIA gene by using deletions that joined the two genes. Also, extensive (− +) frame-shifts within this region did not affect rIIB function.

2. Identification of UGA mutations in the rII mutant collection

The tryptophan (Trp) codon UGG can mutate to UAG (*amber*) or to UGA and thus form 'close pairs' of *amber* and UGA mutants. Close pairs were indeed found in the rII mutant collection; the *ambers* HB74, N97, and X237 mapped close to the mutants X655, X665, and N65 that were neither *ambers* nor *ochres*, and hence were putatively UGAs. Brenner *et al.* initially focused on the rIIB mutant X655 of the HB74/X655 close pair. Although, the X655 mutant was induced by 2-AP, mutants genetically indistinguishable from X655 were also induced by HA, which suggested that the mutant codon must differ from an acceptable (i.e. amino acid inserting) codon by a G-C to A-T change. X655 was not UAG or UAA since it was not *amber*- or *ochre*-suppressible, and 2-AP, but not HA, could revert it to r^+. Thus, either X655 contained no G-C for HA to act upon, or a G-C to A-T change

converted X655 to another unacceptable triplet. Exploring the latter possibility, Brenner *et al.* showed that both 2-AP and HA could convert X655 to an *ochre* (UAA). That is, plaques could be obtained on an *ochre*-suppressor K12 strain but not on non-suppressor or amber-suppressor K12. Moreover, this change did not require phage growth, which suggested that the G to A change must occur in mRNA. Since X655 is not *amber* (UAG), therefore it must be UGA. Additionally, the UGA-derived *ochres* (UAAs) could be converted by 2-AP to *ambers* (UAGs), and the *ambers* obtained in this roundabout way were found to map identically to the HB74 *amber* (i.e., the production of r^+ recombinants was not significantly above the reversion rate, which was 2×10^{-7} to 9×10^{-7}). In contrast, cross-over between HB74 (*amber*) and X655 (UGA) produced r^+ recombinants at frequencies of 2×10^{-5} to 6×10^{-5}, presumably via the restoration of the UGG codon (Trp) in r^+ progeny.

3. Inferring that UGA is non-coding

The first part of rIIB is dispensable, and almost all of the many rII mutants examined in it were frame shift mutations. There were only fifteen base analogue mutants, of which 13 were *ochres* or *ambers;* one was a temperature-sensitive (*ts*) and the remaining one was X655 (UGA). The bias for chain-termination mutations was consistent with the region's dispensability, and suggested that UGA also is a chain terminator. Additionally, as noted above, (+ −) frame shifts can be tolerated in the rIIB first part, but some pairs of complementary frame shift did not suppress each other, presumably because unacceptable triplets formed in the shifted frame acted as barriers to mutual suppression. One barrier was an *amber* (i.e. the barrier was amber-suppressible), two were *ochres* (they were ochre-suppressible), and three were UGA (by their HA-induced conversion to *ochre*). The unacceptability of UGA in X655 and the three barriers was unlikely to reflect amino-acid insertion, and suggested UGA was nonsense. Finally, the *r*1589 deletion, which fuses the *rIIA* and *rIIB* genes to make a hybrid protein containing a nonfunctional N-terminal rIIA fragment fused to a functional C-terminal rIIB fragment, lost its rIIB-complementing ability when combined with the rIIA UGA mutation X665. Presumably, chain termination prevented formation of the hybrid protein. This fulfilled Benzer and Champe's test for nonsense. Together, these findings indicated that UGA was a nonsense codon.

4. Might UGA encode a 'protein inactivating' cys?

Since UGU and UGC encode cys, Brenner *et al.* considered the possibility that UGA might encode a 'protein inactivating' cys. The genetic evidence against this idea was that certain (++) phase shift combinations in the first part of rIIB produced anomalous 'minutes' on K12, indicating a very incomplete restoration of the WT phenotype. The minutes very likely reflect some kind of phase error, and were produced only by (+ +) pairs that straddled the b6 (UGA) barrier. Thus, UGA could not be associated with any normal amino-acid reading, and strongly suggested that it was nonsense. Additional chemical evidence came from the experiments by Khorana *et al.* who showed that poly-(UGA)n used as mRNA in a cell-free system induced the synthesis of polymethionine (corresponding to AUG) and poly-aspartic acid (corresponding to GAU), but no poly-cysteine. Therefore, it was unlikely that UGA encoded cysteine.

5. A bit of historical context

Less than two weeks after the Brenner *et al*. paper appeared in the 4th February issue of *Nature*, an article from the Nirenberg group at the NIH appeared in the 17th February issue of *Science* (Marshall *et al*. 1967). It was not specifically about UGA, but it used biochemistry to ask whether the genetic code was universal. Marshall *et al*. decided that it probably was, but from their results, they decided that UGA coded for cysteine. Katz, Brenner's PhD student, and later my PhD guide, recalls being briefly nervous, but as it turned out 'genetics always trumps biochemistry'. I think it apt to conclude with Katz's words, '… our paper showed, once more, the power of genetics and the rII as a genetic system. We had no idea what the rII gene did, nor did we care.'

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