Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

Why Are Suppressors of Amber Mutations So Frequent Among *Escherichia coli* K12 Strains?: A Plausible Explanation for a Long-Lasting Puzzle

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AMBER mutations, which generate the chain-terminating UAG codon, have played a central role in the development of molecular biology, in particular in deciphering the relationship between DNA and proteins (Brenner *et al.* 1965). These mutations are conditional in the sense that their phenotype is observed only in certain bacterial strains. Amber mutations were independently isolated in several systems in the early 1960s. Seymour Benzer identified ambivalent *rII* mutants of bacteriophage T4 that grew on a subset of *Escherichia coli* K(λ) strains and coined the "sense *vs.* nonsense" terminology (Benzer and Champe 1961). Alan Campbell was looking for additional sites on the bacteriophage λ map to study prophage integration and isolated *su*ppressor *s*ensitive mutations (*sus*) in most essential genes (Campbell 1961). Alan Garen identified "extreme negative" and suppressible mutants of alkaline phosphatase (Garen and Siddiqi 1962). Finally, Richard Epstein and Charles Steinberg were attempting to isolate anti-*rII* mutants of T4 that would grow only on K(λ) strains and instead identified most of the essential genes of T4 (Epstein *et al.* 1964); the background and history of the T4 amber mutants were recently reviewed by Frank Stahl (Stahl 1995).

What made the amber mutations so successful? First, amber UAG codons can be generated in almost any gene by single-base substitutions in any of eight triplets that code for seven different amino acids. Thus, even the λ *cro* gene, encoding one of the smallest proteins with only 66 amino acid residues, contains eight codons related to UAG by a single-base substitution. Second, the suppression efficiency of most su_{am} tRNAs can be extremely high, leading to up to 60% amino acid insertion at the amber codon. Thus, even structural proteins can be synthesized in sufficient amounts to allow normal growth; this is not the case with UAA *ochre* mutants, which are poorly suppressed. Finally, the isolation of temperature-sensitive tRNA suppressors of amber codons (Smith *et al.* 1970) expanded considerably the usefulness of these two major classes of conditional-lethal mutations.

Since UAG is a stop codon in almost 400 *E. coli* genes (http://www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species=Escherichia+coli+K12+[gbbct]), one wonders why it was so easy to identify strains carrying amber-suppressor mutations. Indeed, three of nine derivatives of the original *E. coli* K12 strain harbor three different su_{am} suppressors (Y10, Ymel, and CR63; Bachmann 1987). This suggests that suppression of one or more amber codon(s) not only is neutral for growth, but also may provide an unsuspected selective advantage to a bacterial strain whose growth appears completely normal, at least under standard laboratory conditions. While the purpose of early mutant isolations was mostly focused on the identification of auxotrophs, many of these early strains have been subjected to heavy mutagenesis. This probably accounts for the generation of several different su_{am} suppressor mutations. However, the molecular basis for their fixation remained a puzzle, and a molecular scenario for their appearance has been missing for many years.

The recent characterization of the *rpoS* gene in 13 *E. coli* K12 strains provides a plausible explanation for this puzzle (Atlung *et al.* 2002). Indeed, 6 of these strains carry an UAG amber codon at position 33, while most others have a CAG glutamine codon at this position. In contrast, non-K12 *E. coli* strains and most enterobacteriae have the GAG glutamate codon at this position of the *rpoS* gene. Since transitions are far more frequent than transversions, CAG pseudorevertants are expected to be more prevalent than GAG true revertants. The stability of strains carrying the *rpoS* amber mutation is

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illustrated by the fact that at least two of them, including a derivative of Hfr Cavalli, are devoid of suppressors (Miller 1992). The *rpoS* gene encodes a σ factor required for efficient survival during the stationary phase. RpoS is, for instance, necessary for long-term survival in illuminated seawater, glycogen accumulation, and resistance to H_2O_2. Although *rpoS* mutants exhibit pleiotropic phenotypes under nonoptimal conditions, the gene is not essential in standard laboratory conditions.

It therefore seems likely that at some stage between the isolation of an *E. coli* strain from a convalescent diphtheria patient in 1922 and the establishment of the original K12 strain by E. L. Tatum, the bacterial strain accidentally acquired this amber mutation in *rpoS*. Maintenance of this strain for many years in agar stabs, as well as the heavy mutagenesis used in early experiments, probably provided the selective force for the occurrence and fixation, unnoticed at the time, of the amber suppressors. This fortuitous accident, together with the presence of the F sex factor (Lederberg 1984) and of a λ-prophage (Hershey and Dove 1971), certainly contributed to the amazing success of this particular bacterial strain in the development of molecular biology.

The author thanks Dick Epstein, Costa Georgopoulos, and Patrick Linder for helpful discussions and comments on the manuscript. This work was supported by the State of Geneva and the Fonds National Suisse.

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