Culmination of a half-century quest reveals insight into mutant tRNA-mediated frameshifting after tRNA departure from the decoding site

John F. Atkins[a,b,1]

The pairing of the three anticodon bases of a cognate acylated tRNA to a ribosomal aminoacyl (A)-site three-base codon and the concerted movement of this bound complex into the ribosomal peptidyl (P) site are central to standard nonoverlapping triplet decoding. Subsequently, the then-deacylated tRNA and codon complex occupies the exit (E) site. Accordingly, when a mutant tRNA that permitted a modest level of four-base translocation at a specific four-base mRNA sequence was found to have an extra base (i.e., nucleotide 37.5) in its anticodon loop, it was proposed to engage in quadruplet anticodon:codon pairing (1, 2). This and numerous counterpart tRNA mutants were selected as suppressors that restored some original frame decoding to single-nucleotide insertion or deletion mutants (reviewed in refs. 3 and 4). Because the compensatory mutation was in coding sequence independent of that in which the primary lesion occurred, the tRNA mutants were termed "frameshift-mutant external suppressors." A substantial number of these frameshift suppressors were initially similarly interpreted in terms of potential for expanded anticodon pairing with four A-site mRNA bases (although some were thought to involve occlusion of the fourth mRNA base). However, subsequent genetic and later primer extension work shifted the focus from the theory that codon:anticodon pairing in the A site directly determines the framing switch, to the alternative in which initial triplet A-site pairing is followed by post–A-site anticodon:codon dissociation, with subsequent realignment leading to tRNA anticodon re-pairing to mRNA in a new frame (4, 5). X-ray crystallographic studies on a $tRNA^{Pro}$ anticodon loop with an extra base definitively showed not only a standard 3-nt anticodon (bases 34, 35, and 36) decoding in the original frame codon, but also ribosomal RNA (rRNA) nucleotides enforcing such pairing despite the extra anticodon loop nucleotide (i.e., base 37.5) (6). In standard decoding, four 3′ bases of the anticodon loop form a stacked array, whereas with the mutant tRNA, extra base 37.5 also participates to make it a 5-nt stack (6). The work by Hong et al. (7) provides major insight into the conformational changes and mechanism of the frameshifting after A-site departure.

After translocation to the ribosomal P site, the anticodon is paired to three mRNA nucleotides in the +1 frame. The work by Hong et al. (7) reveals two successive anticodon loop conformational changes after the tRNA is translocated from the A site to the less-constraining internal ribosome environments where anticodon loop structure is minimally monitored. As illustrated in figure 2 of ref. 7, the first remodeling involves the extra anticodon base becoming flipped out of the stack to make a standard-sized four-base stack. In the A site, the extra stacked base forced disruption of the stabilizing cross-anticodon loop pairing between U32 (5′ adjacent to the anticodon) and A38 (second base to the 3′ end of the anticodon). In the restored four-base stack in the P site, U32:A38 pairing is present. The second remodeled form, which is present in an intermediate state between the P and E sites, involves base 37.5 swapping position with that at position 37. The latter then engages with an rRNA nucleotide in a manner proposed by Hong et al. (7) to be significant for the frameshifting mechanism. This involves disruption of rRNA stacking interactions that are thought to stabilize standard framing and, thus, facilitate realigned pairing. Pairing in the new +1 frame results in mRNA scrunching so that seven, rather than six, nucleotides are encompassed within the P- and E-site region (an extra A-site base is known only for eukaryotic protein-mediated termination). Hong et al. (7) propose that upon full translocation of the mutant tRNA to the E site, the 5′ (now-extra) nucleotide exits the E site, facilitating standard decoding in the new frame.

Suppressor Diversity

Although the structural insights now being reported are a major advance, frameshift-mutant suppressors

[a]School of Biochemistry and Cell Biology, University College Cork, Cork T12 YT57, Ireland; and [b]Department of Human Genetics, University of Utah, Salt Lake City, UT 84112

Author contributions: J.F.A. wrote the paper.

The author declares no conflict of interest.

See companion article 10.1073/pnas.1809319115.

[1]Email: john.atkins@genetics.utah.edu.

are diverse. Elucidation of the detailed mechanisms involved in a number of other types involving different translation component mutants is merited. With the mutant tRNA studied by Hong et al. (7), the anticodon nucleotides that re-pair to mRNA in the +1 frame are the same as those involved in the initial original-frame pairing. However, in some other cases, it appears that there is a shift in the anticodon position within the expanded anticodon loop (4). Some suppressors involve an anticodon loop base substitution, a base modification defect, or two extra anticodon loop bases (4). Not surprisingly, given the flexibility of tRNA and its significance during protein synthesis, frameshift-causing tRNA mutations at several positions outside of the anticodon loop have been genetically characterized (4, 5). One of these has 10 extra bases in its 5-methyluridine-pseudouridine-cytidine (TFC) loop (8), and another has, as its sole change, a substitution of the 5′ C of the universally conserved CCA at the 3′ end (i.e., amino acid-acceptor end) of tRNA (9). Single-protein mutants can also cause or enhance frameshifting. The mechanism by which mutants of *hrpA* enhance frameshifting by a substitution of the third base from a tRNA 3′ end is unknown (4, 9). Another challenge involves ribosomal protein bL9. While frameshift-mutant studies have provided supportive evidence for the initial suggestion that bL9 acts as a strut between adjacent ribosomes (10) and constrains forward mRNA slippage due to trailing ribosome pushing (4), more recent data (11, 12) highlight the complexity and importance of further work for a full understanding of ribosome functioning. Truncation of the C-terminal end of protein bS9 that normally makes contact with the 5′ phosphate of tRNA nucleotides 33 and 34 causes frameshifting (5), as do alterations at several relevant positions in rRNA, although, to a moderate extent, mutants of elongation factor thermo unstable (EF-Tu) also enhance frameshifting. Lastly, limitation of the cognate decoder, be it tRNA when there is a sense codon in the A site or a release factor when there is a stop codon in the A site, can lead to frameshifting by the tRNA that decoded the 5′ adjacent codon (4).

An indirect but important consequence of the study of frameshift-mutant suppressors in yeast was that mutants that enhanced suppressor efficiency, *UPF1* and *UPF2* (for up-frameshift), provided an entry point into the study of nonsense-mediated mRNA decay (3). A more direct consequence has been exploration of the potential of four-base codons to provide unassigned codons for synthetic code expansion studies in which desirable novel amino acids are encoded. While a clever scheme was used to isolate ribosome mutants that enhance such decoding by tRNAs with expanded anticodon loops (13), the structural work by Hong et al. (7) relates to the challenges in achieving this goal if high-fidelity triplet decoding is to be maintained at other codons. Transient inactivation of the A-site constraints to allow efficient quadruplet anticodon:codon A-site pairing by mutant tRNAs and achieving highly efficient post–A-site reframing without employing a "special" flanking mRNA sequence (see below) are not simple tasks. While there are a tiny number of natural tRNAs with expanded anticodon loops, there is no evidence that they mediate productively utilized frameshifting.

Natural Frameshifting

The discovery of mutant translation component-mediated frameshifting (14, 15) led quickly to the discovery of error frameshifting by wild-type translation components and the relevant significance of the balance of certain wild-type tRNAs. It then took a considerable amount of time to substantiate the dream that selection had operated so that frameshifting is productively, importantly, and naturally utilized. In different situations, contrasting (although optimal) levels of frameshift were selected, of which some are regulatory sensors and effectors. The features commonly selected were shift-prone sites and features of the flanking mRNA or product encoded 5′ of the shift site. The flanking mRNA features, recoding signals, for this programmed frameshifting include mRNA stem loops, pseudoknots, interaction with certain specific proteins, and pairing of specific mRNA sequence with the rRNA of translating ribosomes (reviewed in refs. 16–18). The latter was also employed in the mutant tRNA structural work by Hong et al. (7). Pertinent also for the Hong et al. work was the earlier proposal that even for programmed frameshifting with an A-site sense codon, the shift in frame was post-A site (19).

The work by Hong et al. provides major insight into the conformational changes and mechanism of the frameshifting after A-site departure.

The initial motivation in searching for frameshift-mutant suppressors was to test the correctness of the then-prevailing view that selection had resulted in single-step immutability and inflexibility of triplet decoding (18). The hope was that, instead, frameshifting was utilized and that single translation component mutants that permitted it at other places would provide insight into code evolution. Relative weakness of triplet codon:anticodon pairing in the absence of ribosome stabilization, infidelity considerations, and difficulties in imaging transitions between different codon sizes without disruption of encoding prior selected products has generated creative suggestions for the origin of decoding (20). The finding that rRNA in translating ribosomes can pair with specific mRNA has raised the issue whether such pairing in protoribosomes may have been relevant to diffusion (18). The anticodon loop base-flipping now characterized in the mutant tRNA structural work by Hong et al. (7) is pertinent to proto-tRNA evolution, with ribosome plasticity also being relevant. The quest for productively utilized programmed frameshifting is ongoing, with single-molecule FRET and structural studies (especially with cryo-EM) now being powerful additions to the technical repertoire. The ability of single-molecule FRET to provide information about the speed of the various stages, very transient states, and relationships to factor arrival and departure times powerfully compliments the cryo-EM studies now underway.

Acknowledgments

The author thanks friends and other collaborators for enabling him to be in a position to write this commentary.

1 Riddle DL, Carbon J (1973) Frameshift suppression: A nucleotide addition in the anticodon of a glycine transfer RNA. *Nat New Biol* 242: 230–234.
2 Roth JR (1981) Frameshift suppression. *Cell* 24:601–602.
3 Culbertson MR (2007) Navigating without a road map. *Genetics* 177:1–7.
4 Atkins JF, Björk GR (2009) A gripping tale of ribosomal frameshifting: Extragenic suppressors of frameshift mutations spotlight P-site realignment. *Microbiol Mol Biol Rev* 73:178–210.

5 Jäger G, Nilsson K, Björk GR (2013) The phenotype of many independently isolated +1 frameshift suppressor mutants supports a pivotal role of the P-site in reading frame maintenance. *PLoS One* 8:e60246.
6 Maehigashi T, Dunkle JA, Miles SJ, Dunham CM (2014) Structural insights into +1 frameshifting promoted by expanded or modification-deficient anticodon stem loops. *Proc Natl Acad Sci USA* 111:12740–12745.
7 Hong S, et al. (2018) Mechanism of tRNA-mediated +1 ribosomal frameshifting. *Proc Natl Acad Sci USA*, 10.1073/pnas.1809319115.
8 Tuohy TM, Li Z, Atkins JF, Deutscher MP (1994) A functional mutant of tRNA(2Arg) with ten extra nucleotides in its TFC arm. *J Mol Biol* 235:1369–1376.
9 O'Connor M, Willis NM, Bossi L, Gesteland RF, Atkins JF (1993) Functional tRNAs with altered 3′ ends. *EMBO J* 12:2559–2566.
10 Hoffman DW, et al. (1994) Crystal structure of prokaryotic ribosomal protein L9: A bi-lobed RNA-binding protein. *EMBO J* 13:205–212.
11 Naganathan A, Wood MP, Moore SD (2015) The large ribosomal subunit protein L9 enables the growth of EF-P deficient cells and enhances small subunit maturation. *PLoS One* 10:e0120060.
12 Fischer N, et al. (2015) Structure of the *E. coli* ribosome-EF-Tu complex at <3 Å resolution by Cs-corrected cryo-EM. *Nature* 520:567–570.
13 Chin JW (2017) Expanding and reprogramming the genetic code. *Nature* 550:53–60.
14 Riyasaty S, Atkins JF (1968) External suppression of a frameshift mutant in *Salmonella*. *J Mol Biol* 34:541–557.
15 Yourno J, Tanemura S (1970) Restoration of in-phase translation by an unlinked suppressor of a frameshift mutation in *Salmonella typhimurium*. *Nature* 225:422–426.
16 Dinman JD (2012) Mechanisms and implications of programmed translational frameshifting. *Wiley Interdiscip Rev RNA* 3:661–673.
17 Firth AE, Brierley I (2012) Non-canonical translation in RNA viruses. *J Gen Virol* 93:1385–1409.
18 Atkins JF, Loughran G, Bhatt PR, Firth AE, Baranov PV (2016) Ribosomal frameshifting and transcriptional slippage: From genetic steganography and cryptography to adventitious use. *Nucleic Acids Res* 44:7007–7078.
19 Weiss RB, Dunn DM, Shuh M, Atkins JF, Gesteland RF (1989) *E. coli* ribosomes re-phase on retroviral frameshift signals at rates ranging from 2 to 50 percent. *New Biol* 1:159–169.
20 Noller HF (2011) Evolution of protein synthesis from an RNA World. *RNA Worlds: From Life's Origins to Diversity in Gene Regulation*, eds Atkins JF, Gesteland RF, Cech TR (Cold Spring Harbor Lab Press, Cold Spring Harbor, NY), pp 141–154.