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

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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 RNA 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 dissociation, with subsequent realignment leading to tRNA anticodon re-pairing to mRNA in a new frame (4, 5). X-ray crystallographic studies on a tRNA37.5 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

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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 anti-
codon 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
two 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
hpA 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 pro-
vided 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 bL9 that normally makes contact
with the 5′ phosphate of tRNA nucleotides 33 and 34 causes fra-
meshifting (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 en-
hanced suppressor efficiency, UPF1 and UPF2 (for up-frameshifting),
provided an entry point into the study of nonsense-mediated
mRNA decay (3). A more direct consequence has been explora-
tion 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 ex-
panded anticodon loops, there is no evidence that they mediate
productively utilized frameshifting.

Natural Frameshifting
The discovery of mutant translation component-mediated fra-
meshifting (14, 15) led quickly to the discovery of error frame-
shifting 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

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