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Minireview

Making the jump: new insights into the mechanism of *trans*-translation

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Abstract

The transfer-messenger ribonucleoprotein (tmRNP), which is composed of RNA and a small protein, small protein B (SmpB), recycles ribosomes that are stalled on broken mRNAs lacking stop codons and tags the partially translated proteins for degradation. Although it is not yet understood how the ribosome gets from the 3' end of the truncated message onto the messenger portion of the tmRNA to add the tag, a recent study in *BMC Biology* has shed some light on this astonishing feat.

Discovery and properties of transfer messenger RNA

tmRNA was discovered in 1995 [1], when Simpson and co-workers overexpressed a mouse cytokine in *Escherichia coli* and found truncated cytokine peptides each tagged at the carboxyl termini with the same 11-amino acid residue extension AANDENYALAA. This tag sequence turned out to be encoded in a small stable RNA that had been identified many years earlier as a 10S RNA of unknown function [2]. The 10S RNA is now known as transfer messenger RNA (tmRNA). As its name implies, tmRNA has features of both transfer RNA and messenger RNA. One domain of the molecule, known as the transfer RNA-like domain (TLD), has an amino acid acceptor stem chargeable with alanine and a T arm with modified nucleotides, just as in tRNA (Figure 1). However, the D arm of the tRNA-like domain is degenerated, and there is no anticodon loop. A second domain, the mRNA-like domain (MLD), is located in a pseudoknot-rich region and contains a short open reading frame that encodes AANDENYALAA and is followed by a normal stop codon. It was quickly established that this peptide targets the truncated ribosomal product for degradation [3].

These observations led to the proposal that the tmRNA occupies the empty A site of the stalled ribosome which then jumps or slides from the 3′ end of the truncated message onto the MLD, at a triplet known as the resume codon (in *E. coli* this is a GCA triplet) from where translation continues normally until an in-frame tmRNA stop codon is encountered (Figure 2). This process is known as *trans*-translation [3]. In nature, bacteria use this seemingly complicated trick to proteolytically destroy proteins that are synthesized from damaged mRNA templates and, perhaps more importantly, to reactivate and recycle needed ribosomes [4]. In some bacteria, the gene for tmRNA (*ssrA*) is essential [5-7], but in other species *trans*-translation is important only to survive challenging environmental growth conditions, and this is probably the reason for the relatively late discovery of this fundamental capability of every bacterial cell.

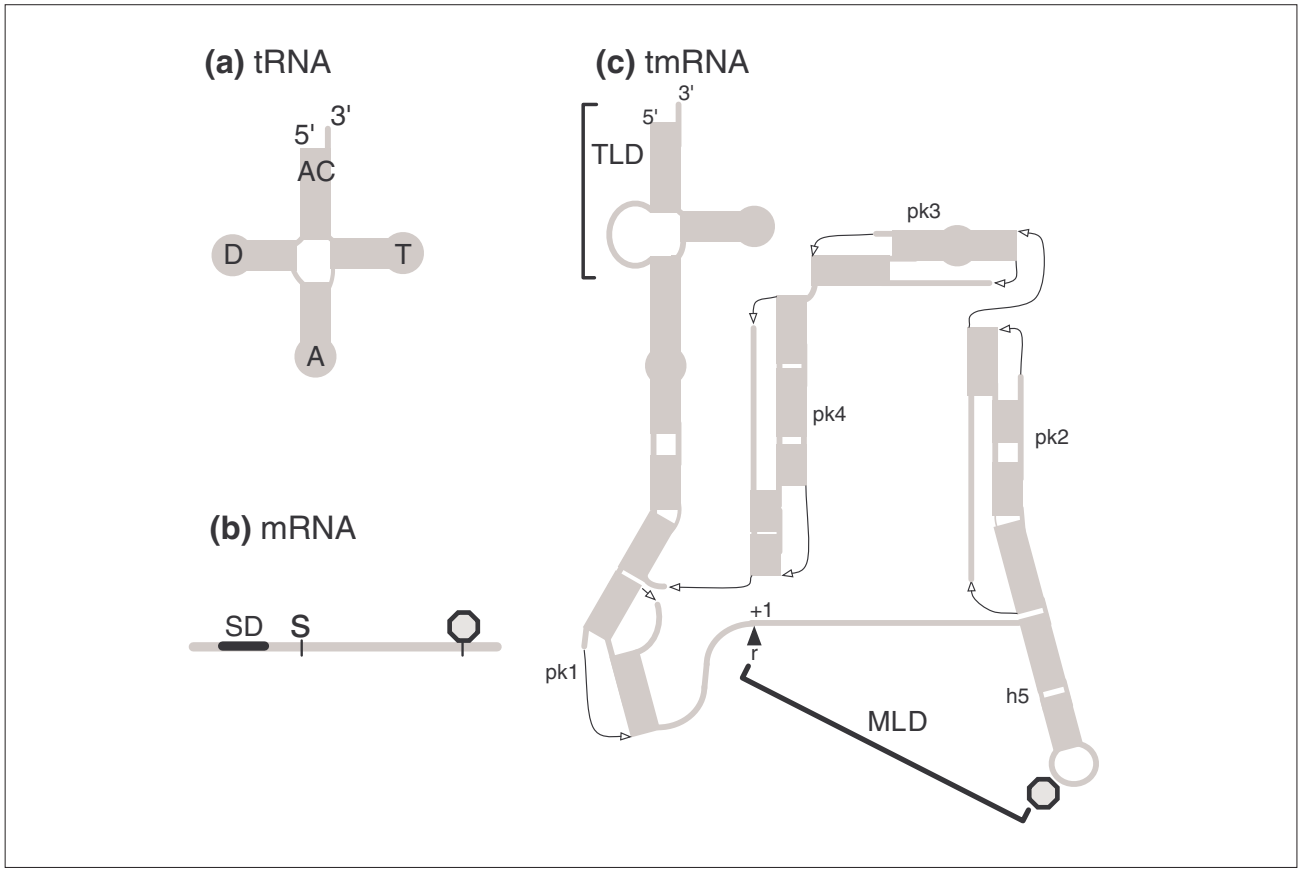

Figure 1
Comparison of the structures of **(a)** tRNA, **(b)** mRNA and **(c)** tmRNA. (a,c) The 3' and 5' termini, the amino acid acceptor stem (AC) and the anticodon (A), D and T arms are indicated. (b,c) The Shine-Dalgarno sequence (SD), the start codon (s) and the stop codon (octagon), the locations of the tRNA-like (TLD) and mRNA-like domains (MLD) as well as pseudoknots (pk) 1 to 4, helix 5 (h5), and the +1 resume codon (r) are indicated. The thin arrows depict the pseudoknot connections.

The mechanism of *trans*-translation however is mysterious. Because the TLD of tmRNA has no anticodon, it is not clear how it can recognize and bind to the empty A site of a stalled ribosome (Figure 2). Moreover, the MLD has neither an AUG start codon nor the Shine-Dalgarno sequence whereby bacterial mRNA binds to a complementary region of the ribosomal RNA at the start of translation. How then is the resume triplet properly positioned? And what mechanism allows the ribosome to take off from the damaged mRNA template and land precisely on the tmRNA's resume codon? Astonishingly, the ribosome performs this feat when a peptide bond forms between the partially synthesized protein and the alanine-charged tmRNA, and while establishing the correct reading frame for continuing elongation. Miller and colleagues [8] have now carried out a systematic site-directed mutagenesis study in an attempt to establish the contribution of the nucleotide residues that precede the resume codon to the correct positioning of the MLD.

Identifying determinants of template switching

One problem in determining the critical elements of *trans*-translation *in vivo* has been that *E. coli* cells grow well without the *ssrA* gene, so mutations cannot be detected by their effects on growth. Furthermore, the tagged proteins produced by *trans*-translation are degraded, and therefore cannot be used to indicate whether it is occurring normally. Luckily, however, a wide variety of tag templates are tolerated, and, upon removal of the natural stop codons, large additions can be engineered onto the tmRNA and are then translated [9]. The group of Allen Buskirk has used an ingenious assay in which proper tagging of truncated kanamycin resistance (KanR) gene products on stalled ribosomes produces full-length KanR protein, so that *E. coli* survives on kanamycin plates only when the tmRNP is functional [10].

The nucleotides surrounding the resume codon have been the focus of several studies aimed at determining what

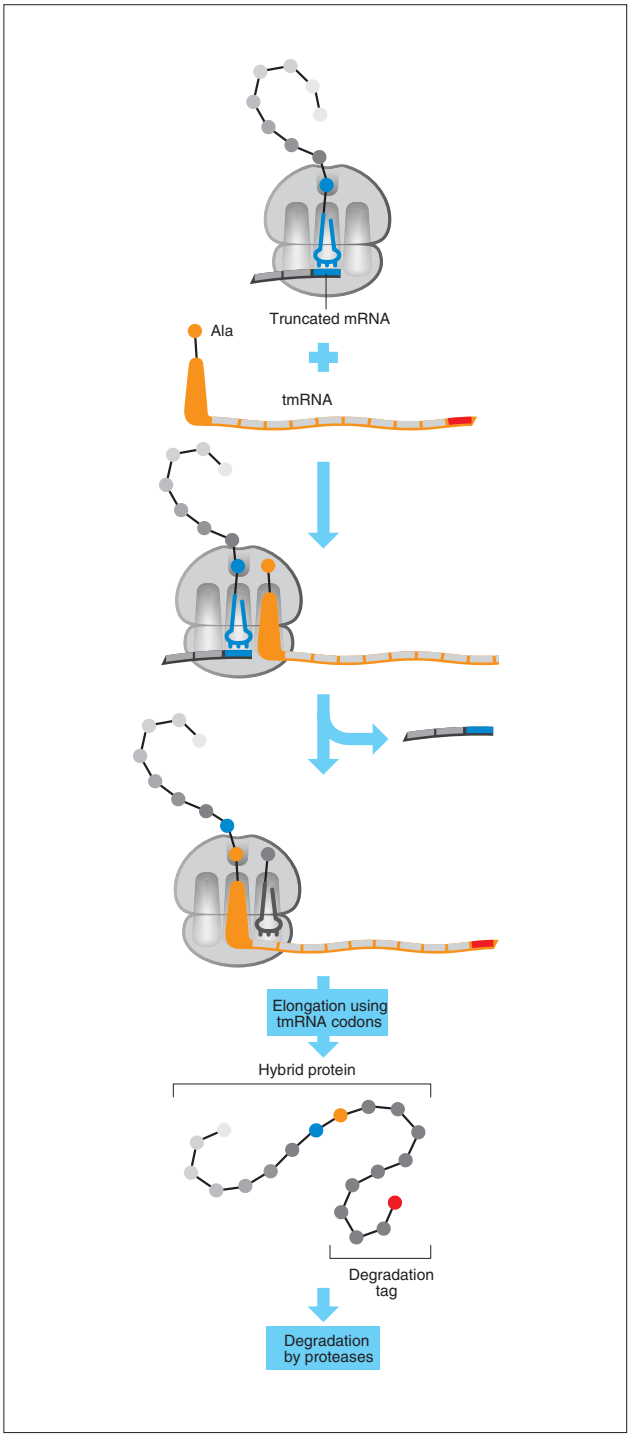

Figure 2
Steps in *trans*-translation. A ribosome remains stalled near the 3' end of broken mRNA, binds to alanine-charged tmRNA (orange), and switches from the broken message onto the open reading frame of the tmRNA allowing regular translation to resume. Upon reaching the tmRNA stop codon, the ribosome releases a hybrid protein with a degradation tag and joins the pool of active ribosomes.

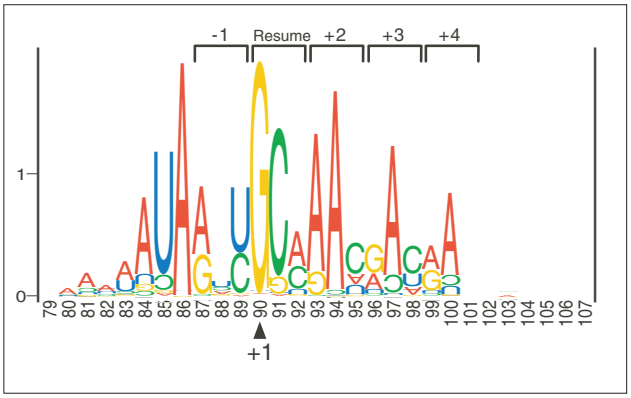

Figure 3
RNA structure logo [19] displaying the information content surrounding the tmRNA resume codon. The height of each symbol is proportional to its frequency in 486 representative sequences from an alignment of 730 tmRNAs [20]. Residues are numbered in reference to *E. coli* tmRNA [21]. The resume codon (+1), codons +2 to +4 and the -1 triplet are indicated.

enables the ribosome to switch templates (reviewed in [11]). The upstream region contains an adenosine-rich cluster of about seven residues adjacent to three nucleotides (the -1 triplet) immediately preceding the +1 guanosine. Downstream of the resume triplet, for unknown reasons, codons +2 to +4 prefer adenosine at the second position (Figure 3). On the basis of sequence comparisons and the idea that the -1 triplet (GUC, at positions 87-89 of *E. coli* tmRNA, Figure 3) should be in the A conformation for allowing tmRNA to participate in the ribosomal elongation cycle, it was proposed that the -1 triplet has a crucial role in template switching. Specifically, if the A conformation is required, 18 out of the 64 theoretically possible -1 triplets are prohibited, so they would yield tmRNAs that could not function in *trans*-translation [12].

The new systematic *in vivo* study from the Buskirk laboratory that has recently been published in *BMC Biology* [8] provides strong experimental evidence that the previously suspected -1 resume triplet has only a minor role in accommodating tmRNA on the ribosome. In this paper, Miller and colleagues [8] constructed mutant tmRNAs with all 64 possible permutations of the -1 triplet and determined their effect on survival in the kanamycin resistance assay. They found that eight of the 18 codons that were prohibited according to the -1 hypothesis [12] were in fact fully functional, and other mutant tmRNAs that were predicted by the -1 triplet rule to be functional were shown by experiment to be completely inactive. The results of this comprehensive study show that the proposed rule for the -1 triplet is invalid and suggest different nucleotides that are important for accommodation of tmRNA on the ribosome.

One alternative nucleotide is the highly conserved adenosine at position 86 of *E. coli* tmRNA (Figure 3), which was observed earlier to be important in *trans*-translation [13]. Indeed, by measuring survival in the kanamycin-resistance assay, the investigators confirmed that changing A86 to a pyrimidine yielded cells that were unable to *trans*-translate.

Because high-resolution structures of the ribosome-bound tmRNA at various stages of *trans*-translation are currently unavailable, it is unclear why the conserved A86 has such a prominent role. Although this adenosine residue may act independently to interact with the ribosome, the investigators suggest that the A86 interacts with a yet to be identified ligand that is primarily responsible for engaging the resume triplet and tmRNA in the attachment and synthesis of the tag peptide. They speculate that A86 might bind to the SmpB that is part of the transfer-messenger RNA ribonucleoprotein, or to ribosomal protein S1, two proteins that have been found by other investigators to be close to the decoding center of the ribosome-bound tmRNA at some stage of *trans*-translation [14-18]. Further studies at the atomic level will be required before the athletic potential of the ribosome is fully understood.

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