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Bifunctional transfer-messenger RNA

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Abstract

Transfer-messenger RNA (tmRNA) is a bifunctional RNA that has properties of a tRNA and an mRNA. tmRNA uses these two functions to release ribosomes stalled during translation and target the nascent polypeptides for degradation. This concerted reaction, known as *trans*-translation, contributes to translational quality control and regulation of gene expression in bacteria. tmRNA is conserved throughout bacteria, and is one of the most abundant RNAs in the cell, suggesting that *trans*-translation is of fundamental importance for bacterial fitness. Mutants lacking tmRNA activity typically have severe phenotypes, including defects in viability, virulence, and responses to environmental stresses.

tmRNA

Transfer-messenger RNA (tmRNA), also called 10Sa or SsrA RNA, is unique among bifunctional RNAs in that it has properties of a tRNA and an mRNA. The ends of tmRNA fold into a structure resembling tRNA(Ala). However, tmRNA is significantly larger than a tRNA, and in place of the anticodon loop there are multiple pseudoknots and a specialized open reading frame. This unusual structure allows tmRNA to interact with specific ribosomes in a reaction known as *trans*-translation. During *trans*-translation, tmRNA performs functions of both a tRNA and a message to induce the ribosome to add a peptide tag to the C terminus of the nascent polypeptide. This reaction targets the nascent polypeptide for degradation and releases the ribosome. Although the physiological role of *trans*-translation is still under investigation, tmRNA has been found in every bacterial genome sequence and is one of the most abundant RNAs in the cell, suggesting that it confers a strong evolutionary advantage in all environments that support bacterial life.

tmRNA structure – tRNA with a message

The 5' and 3' ends of tmRNA fold into a structure containing an acceptor stem and a TΨC arm (Fig. 1). The acceptor stem contains a G:U wobble base pair, which allows it to be recognized by alanyl-tRNA synthetase and charged with alanine [1-3]. The tRNA-like domain is bound by two proteins that are essential for *trans*-translation activity: SmpB, a dedicated component of the *trans*-translation system, and EF-Tu, which is important for functional interactions with the ribosome [4-6].

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The tag reading frame encodes a short peptide and is contained within an extended (>250 nt) sequence that includes 2-4 pseudoknots, depending on the species [7, 8]. Although this sequence is translated during *trans*-translation, it lacks a canonical start codon and there is no indication that ribosomes can initiate translation on the tag reading frame. Therefore, it is not a true open reading frame. Annotated tag reading frames range in length from 8-35 codons, and terminate in a stop codon [8]. However, the tag reading frame can be shortened to one codon or extended to encode complete proteins without disrupting *trans*-translation activity. The role of the pseudoknots in this region is unclear. *E. coli* tmRNA is still functional if any of the pseudoknots is replaced, so none of these elements is essential for activity [9, 10]. However, the evolutionary conservation of the pseudoknots, particularly PK1, suggests that they have a role *in vivo*.

***trans*-Translation – a concerted reaction for bifunctional RNA**

Unlike other bifunctional RNAs described in this issue, which can use their different functions independently, the tRNA-like and mRNA-like functions of tmRNA are used in a single, concerted reaction (Fig. 2) [11]. tmRNA, charged with alanine and bound to SmpB and EF-Tu, can enter the A site of substrate ribosomes acting like a tRNA. Cryo-electron microscopy studies and chemical probing experiments indicate that the tRNA-like domain of tmRNA is located at the peptidyl transfer center, with SmpB in the position normally occupied by anticodon loop of a tRNA [12, 13]. The nascent polypeptide is then transpeptidated to alanyl-tmRNA, and the tmRNA-SmpB complex is translocated to the ribosomal P site. At a still undefined point in this process, the mRNA that was being translated is released from the ribosome, and the tag reading frame of tmRNA is inserted into the decoding center. Translation resumes using the tag reading frame as a message, so during decoding of the first codon of the tag reading frame tmRNA is simultaneously mimicking both a tRNA and an mRNA. Translation termination at a stop codon at the end of the tag reading frame completes *trans*-translation by releasing the tagged protein and the ribosome [11]. The tmRNA-encoded peptide tag is recognized by several intracellular proteases, ensuring that the tagged protein is rapidly degraded [11, 14-16].

The tRNA-mimicking functions during *trans*-translation are accomplished by a 1:1 complex of tmRNA and SmpB [4, 12, 17]. SmpB is an essential component of the *trans*-translation machinery, and like tmRNA, SmpB has been found in all bacterial genome sequences [4, 8]. In addition to its role as a structural mimic of a tRNA anticodon loop within the ribosome, SmpB is important for the stability of tmRNA *in vivo*, and makes specific contacts with alanyl-tRNA synthetase to enhance the rate of aminoacylation of tmRNA [18, 19].

The mRNA-mimicking functions of tmRNA seem straightforward, but tmRNA faces two challenges not encountered by typical mRNAs. First, tmRNA does not have a start codon or canonical initiation sequences, and translation will not initiate on the tag reading frame. Instead, during *trans*-translation the tmRNA-SmpB complex directs translation to resume at a specific codon at the beginning of the tag reading frame. Correct recognition of this “resume codon” by the ribosome involves residues immediately upstream of the translated sequence, as well as SmpB [20-22]. The second challenge for the mRNA-like function of tmRNA is to thread the tag reading frame through the mRNA channel despite the significant secondary structure and surrounding pseudoknots. Despite the apparent structural constraints imposed by the pseudoknots, biochemical and cryo-EM studies have suggested that the pseudoknots can remain intact when the tag reading frame is in the mRNA channel [23-25].

Physiology of *trans*-translation

trans-Translation occurs frequently in growing cells. Estimates of tagging frequency in *E. coli* suggest that about 0.4% of translation reactions end in *trans*-translation [26]. This rate

corresponds to approximately one *trans*-translation reaction per ribosome per cell cycle. There appear to be two distinct roles for *trans*-translation: translational quality control, and gene regulation. When translational complexes stall due to mRNA damage or other problems completing translation, *trans*-translation is used to remove all components of the problematic complex: the stalled ribosome is released at the tag reading frame stop codon, the nascent polypeptide is targeted for proteolysis by the tmRNA-encoded tag, and the mRNA is removed from the ribosome and degraded. In other cases, translation complexes are intentionally targeted to tmRNA during expression of specific genes, and *trans*-translation serves to control the amount of protein that is produced.

Both translational quality control and gene regulation by *trans*-translation depend on the selectivity of tmRNA for stalled translation complexes. In vitro, tmRNA-SmpB interacts efficiently with ribosomes that are at or near the 3' end of an mRNA, but *trans*-translation is blocked if the mRNA extends past the leading edge of the ribosome [27]. Likewise, most if not all substrates for *trans*-translation in vivo result from ribosomes that are stalled near the 3' end of the mRNA [28, 29]. How tmRNA recognizes these stalled complexes is not yet known, but it has been proposed that either mRNA extending past the leading edge of the ribosome sterically blocks tmRNA entry into the ribosome, or that the ribosome changes conformation when it reaches the end of the mRNA and tmRNA recognizes the altered conformation [29]. Experiments in *E. coli* demonstrated that *trans*-translation does not compete with translation elongation, confirming that tmRNA only acts on selected translation complexes in vivo [26].

Why would a ribosome translate to the 3' end of an mRNA without terminating? Part of the answer is that in bacteria ribosomes may frequently initiate translation on truncated mRNAs. Eukaryotes have several mechanisms to ensure an mRNA is complete before a ribosome initiates translation, including nuclear export controls and recognition of the poly(A) tail by translation initiation factors [30]. However, in bacteria translation can initiate before transcription is complete, so there is no opportunity to proofread the mRNA or insure that it has a correct 3' end. If RNA polymerase terminates before the stop codon is transcribed, the mRNA will have no translation termination signal. When truncated mRNAs are produced intentionally by inserting a transcriptional terminator before the stop codon, *trans*-translation acts efficiently on the resulting translation complexes [11]. Likewise, if the mRNA is truncated by physical or chemical damage, or by nucleolytic degradation, the stop codon can be removed. For example, the endotoxin protein RelE cuts mRNAs within the ribosome, causing *trans*-translation [31, 32]. DNA damage can also lead to premature transcription termination. In *E. coli*, tmRNA mutants are hypersensitive to 5-azacytidine, a crosslinking agent that blocks transcription, suggesting that tmRNA aids in elimination of stalled translation complexes at these sites [33]. Ribosomes can also reach the end of an intact mRNA due to readthrough of the stop codon, and both suppressor tRNAs and antibiotics that promote miscoding and frameshifting increase the amount of *trans*-translation [34, 35].

Ribosomes stalled in the middle of an mRNA can also be targeted for *trans*-translation if the 3' portion of the mRNA is degraded. Stalling due to scarcity of a cognate tRNA or release factor, or due to an inefficient translation termination sequence, leads to truncation of the mRNA [36-42]. In at least some cases, the truncation is due to degradation of the mRNA by RNase II exonuclease, which appears to facilitate cleavage of the mRNA in the ribosomal A site by a second ribonuclease activity [43]. Other stalled ribosomes, such as those engaged in attenuation or secretion control, are protected from tmRNA activity [44]. Problems with nascent polypeptide folding can also target the translation complex to tmRNA [45]. Although some of the mechanisms for promoting *trans*-translation remain to be elucidated, it appears that the majority of tmRNA activity is used to remove problematic translation complexes and target the incomplete nascent polypeptides for degradation.

tmRNA activity is also used to regulate expression of specific genes (Fig. 3). For example, in *E. coli* the ArfA protein appears to promote release of stalled ribosomes in a pathway that is partially redundant with tmRNA, and the expression of ArfA is controlled by tmRNA activity [46]. The *arfA* mRNA has an RNase III cleavage site before the stop codon, and the mRNA is present predominantly in a truncated form. When tmRNA is available, ArfA is tagged and proteolyzed, and there is little ArfA protein in the cell. However, when tmRNA is absent, a truncated but active ArfA protein is produced [46]. Presumably this mechanism allows ArfA to be produced exclusively when tmRNA becomes saturated and the cell requires more ribosome release activity. Consistent with this idea, deletion of the genes encoding ArfA and tmRNA is synthetically lethal in *E. coli* [47]. The recent discovery of this mechanism raises the possibility that other key proteins are regulated by tmRNA through specific RNase cleavage sites. Expression of other genes, such as *kinA* in some strains of *B. subtilis*, are controlled by tmRNA because the gene contains a transcriptional terminator 5' of the first in-frame stop codon [48] (Fig. 3).

A second mechanism for regulating gene expression with tmRNA activity is through DNA binding proteins that block transcription elongation. In *E. coli*, when Lac repressor (LacI) accumulates to high concentrations it binds an auxiliary operator, *O*₃, which overlaps the coding sequence of *lacI*. This binding blocks RNA polymerase and prevents the mRNA from being completed. tmRNA activity will target LacI made from these truncated mRNAs for proteolysis, preventing excess expression of *lacI*. *E. coli* mutants that lack tmRNA are slow to respond to signals that inactivate LacI, suggesting that excess LacI has accumulated [49]. Similarly, the CcpA protein in *B. subtilis* binds within the coding sequence of *TreP*, causing a transcriptional roadblock and promoting tmRNA activity on ribosomes producing new *TreP* [50]. Mutants that lack the bifunctional activity of tmRNA typically have severe phenotypes. *trans*-Translation activity is essential in *Neisseria gonorrhoea*, *Shigella flexneri*, *Haemophilus influenzae*, and species of *Mycoplasma* [51-54]. *E. coli* can survive without tmRNA as long as they retain the ArfA backup system, but mutants lacking tmRNA activity still have a variety of stress phenotypes [47]. tmRNA is also required for virulence in *Salmonella enterica* and *Yersinia pestis*, and is required for cell cycle control in *Caulobacter crescentus* [55-57].

Regulation of tmRNA activity

tmRNA is abundant in growing cells, but it is further induced by stress conditions such as heat shock, cold shock, and biofilm formation [58-60]. More extensive regulation of tmRNA has been observed in *C. crescentus*, where the abundances of tmRNA and SmpB change as a function of the cell cycle [61]. tmRNA and SmpB levels increase in late G1 phase, and are rapidly removed from the cell after DNA replication initiates. tmRNA is stable in G1-phase cells, but is specifically degraded in early S phase by the exonuclease RNase R [62]. The ability to regulate tmRNA stability may be enhanced in *C. crescentus* by the structure of tmRNA. The gene encoding tmRNA in *C. crescentus* contains a circular permutation, resulting in a mature tmRNA that is composed of two RNA chains [63]. RNase R recognizes the non-tRNA-like 3' end of tmRNA, so the two-piece construction of tmRNA is important for regulated degradation [62]. All α -proteobacteria, as well as at least two other bacterial lineages, also have circularly permuted tmRNA [64]. tmRNA and SmpB are also regulated by subcellular localization in *C. crescentus*. Fluorescence *in situ* hybridization and immunofluorescence data show that tmRNA and SmpB are localized in a helical pattern within the cell [65]. RNase R is localized in a non-overlapping pattern, suggesting that localization may be used in part to limit access of RNase R to tmRNA and prevent inappropriate degradation [65]. The role of localization in *trans*-translation activity is not yet clear.

Despite the conservation of tmRNA throughout bacteria and the requirement for *trans*-translation in a variety of bacterial systems, no bifunctional RNA similar to tmRNA has been found in the nuclear genome of a eukaryote. The requirement for translation quality control may be lower in eukaryotes due to availability of the mRNA proof-reading mechanisms described above. Nevertheless, a protein-based mechanism analogous to *trans*-translation has been identified in yeast. In *Saccharomyces cerevisiae*, proteins translated from aberrant mRNA are ubiquitinated by a dedicated E3 ligase to induce degradation by the proteasome [66]. Therefore, eukaryotes may have evolved proteins to take the place of the bifunctional tmRNA.

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Highlights

- Transfer-messenger RNA (tmRNA) has properties of a tRNA and an mRNA.
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- *trans*-translation contributes to translational quality control and regulation of gene expression.
- tmRNA is conserved throughout bacteria, and is one of the most abundant RNAs in the cell
- Mutants lacking tmRNA activity have severe phenotypes.

Figure 1. Structure of tmRNA

(A) Model of the secondary structure of tmRNA based on base pair covariation and chemical probing data. The tRNA-like domain, tag reading frame (green), and pseudoknots (PK) are indicated. The G:U base pair required for alanylation of tmRNA is shaded. (B) Atomic model of the tmRNA-SmpB complex during *trans*-translation (PDB file 3IYR) [23]. tmRNA (blue) and SmpB (purple) combine to mimic a tRNA and an mRNA.

Fig. 2. Model of *trans*-translation

During *trans*-translation, tmRNA, bound to SmpB (purple square) and EF-Tu (not shown) and charged with alanine (orange square), enters the A site of a stalled translation complex and accepts the nascent polypeptide in a transpeptidation reaction. The mRNA (blue) is released and degraded, and translation resumes on the tmRNA tag reading frame (green). Translation terminates at the tag reading frame stop codon, which is recognized by a release factor (red rectangle), releases the ribosomal subunits and the tagged protein. The tmRNA-encoded peptide tag (green squares) targets the protein for proteolysis.

Fig. 3. Regulation of gene expression by tmRNA

Three mechanisms for intentionally generating an mRNA with no stop codon are shown, and the fate of protein translated from these mRNAs in the presence and absence of tmRNA activity is indicated. *arfA* mRNA from *E. coli* is cleaved by RNase III, removing the in-frame stop codon (red hexagon) [46]. The *kinA* gene in some strains of *Bacillus subtilis* has a transcriptional terminator upstream of the first in-frame stop codon, so the *kinA* mRNA will not have a stop codon [48]. Binding of LacI (magenta ovals) protein to the O_3 operator site blocks transcription of the 3' end of the *lacI* gene, producing an mRNA with no stop codon [49]. In all three cases, tmRNA activity results in tagging and degradation of newly-synthesized protein, but in the absence of tmRNA activity a truncated but active variant of the protein will be released.