Biochimie xxx (2014) 1–11

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Review

Ribosome rescue systems in bacteria

Hyouta Himeno [a, *], Nobukazu Nameki [b, **], Daisuke Kurita [a], Akira Muto [a], Tatsuhiko Abo [c, ***]

[a] Department of Biochemistry and Molecular Biology, Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki 036-8561, Japan
[b] Division of Molecular Science, Graduate School of Science and Technology, Gunma University, 1-5-1, Tenjin-cho, Kiryu-shi, Gunma 376-8515, Japan
[c] Department of Biological Science, Graduate School of Natural Science and Technology, Okayama University, Okayama 700-8530, Japan

ARTICLE INFO

Article history:
Received 31 August 2014
Accepted 15 November 2014
Available online xxx

Keywords:
tmRNA
SmpB
ArfA
YaeJ
Ribosome rescue

ABSTRACT

Ribosomes often stall during protein synthesis in various situations in a cell, either unexpectedly or in a programmed fashion. While some of them remain stalled for gene regulation, many are rescued by some cellular systems. Ribosomes stalled at the 3′ end of a truncated mRNA lacking a stop codon (non-stop mRNA) are rescued by *trans*-translation mediated by tmRNA (transfer-messenger RNA) and a partner protein, SmpB. Through *trans*-translation, a degradation tag is added to the C-termini of truncated polypeptides from a truncated mRNA to prevent them from accumulation in the cell. *Trans*-translation has crucial roles in a wide variety of cellular events, especially under stressful conditions. The *trans*-translation system is thought to be universally present in the bacterial domain, although it is not necessarily essential in all bacterial cells. It has recently been revealed that two other systems, one involving a small protein, ArfA, with RF2 and the other involving YaeJ (ArfB), a class I release factor homologue, operate to relieve ribosome stalling in *Escherichia coli*. Thus, many bacterial species would have multiple systems to cope with various kinds of stalled translation events.

1. Introduction

Since translation is a pivotal event in cells, the mechanism of translation as well as its machineries is elaborately designed. Usually translation proceeds smoothly, although it is often stalled. While some of the ribosome stallings are programmed [1], many of them might be unexpected. Assuming that the coding region of an mRNA is cleaved by nucleases during translation, translation would neither continue nor be terminated because neither aminoacyl-tRNA nor peptide release factor functions in the A-site of the ribosome stalled at the 3′ end of the truncated mRNA lacking a stop codon (non-stop mRNA). Accumulation of such stalled ribosomes would result in shortage of ribosomes in the cell. It has been shown that tmRNA (also known as 10Sa RNA or SsrA RNA), a stable RNA that is 300–400 nucleotides in length, rescues the stalled ribosome on a non-stop mRNA through its actions as a tRNA and an mRNA, and this irregular translation involving co-translational mRNA swapping was named *trans*-translation. During *trans*-translation, SmpB, a tmRNA binding protein, plays a crucial role by compensating for the lack of the lower half of the L-shaped tRNA structure including the anticodon in tmRNA [2,3]. As far as we know, the *trans*-translation system exists in all bacteria, and it is essential in some but not all bacteria. Recently, two other ribosome rescue systems have been revealed: one is mediated by a protein called ArfA with the help of RF2 [4,5] and the other is mediated by a homologue of RF (peptide release factor) called YaeJ or ArfB [6,7] (Fig. 1). Several other rescue factors have also been reported (Table 1). Here, we review the ribosome rescue systems in bacteria by focusing on *trans*-translation, ArfA and YaeJ (ArfB).

2. *Trans*-translation

2.1. tmRNA and trans-translation

Trans-translation is the most extensively studied ribosome rescue system. Initially, tmRNA was found to have a partial structure and function of tRNA: it has an upper-half of the cloverleaf-like secondary structure, and it has capacities for aminoacylation with

* Corresponding author. Tel.: +81 172 39 3592.
** Corresponding author. Tel./fax: +81 277 30 1425.
*** Corresponding author. Tel.: +81 86 251 7862; fax: +81 86 251 7876.
E-mail addresses: himeno@cc.hirosaki-u.ac.jp (H. Himeno), nameki@gunma-u.ac.jp (N. Nameki), tabo@okayama-u.ac.jp (T. Abo).

http://dx.doi.org/10.1016/j.biochi.2014.11.014
0300-9084/

Please cite this article in press as: H. Himeno, et al., Ribosome rescue systems in bacteria, Biochimie (2014), http://dx.doi.org/10.1016/j.biochi.2014.11.014

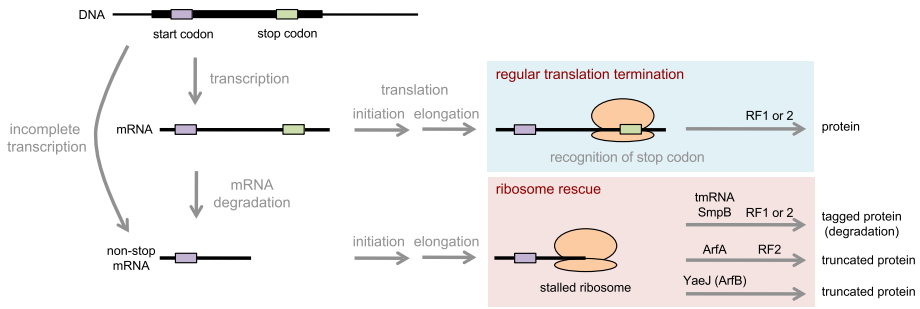

Fig. 1. Three ribosome rescue systems in *E. coli*. Authentic translation termination and three ribosome-rescue pathways underscored in this review are illustrated. In tmRNA-SmpB-driven *trans*-translation, RFs mediate regular translation termination at the stop codon located at the end of the coding region of tmRNA. The nascent polypeptide receives a tmRNA-derived degradation tag and will be degraded. ArfA requires RF2 to rescue the stalled ribosomes. YaeJ (ArfB) is a stop codon-independent RF and rescues the stalled ribosomes by itself. Neither the ArfA/RF2- nor YaeJ (ArfB)-driven ribosome rescue system leads the nascent polypeptides to rapid degradation. Ribosome stalling at the 3′ end of mRNA may also occur via several other events such as readthrough of stop codons or frameshifting [81,132] (not shown in this figure).

alanine by alanyl-tRNA synthetase [8,9] and binding to the ribosome [9,10]. Thereafter, tmRNA was found to have an mRNA function *in vivo* [11] and *in vitro* [12,13]. Keiler et al. [11] have shown that a small peptide of 11 amino acid residues called tag-peptide, the last 10 residues of which are encoded by *Escherichia coli* tmRNA [14], are fused to the C-termini of truncated polypeptides from an engineered, 3′ truncated mRNA having no stop codon. Tag-peptide is synthesized from tmRNA *in vitro* using *E. coli* cell extracts only when it is coupled with poly(U)-dependent poly(Phe) synthesis [13]. A point mutation that inactivates the aminoacylation capacity of tmRNA damages its capacity for tag-peptide synthesis *in vitro*, suggesting that the tRNA function of tmRNA is a prerequisite for its mRNA function [13]. Alanine as the first amino acid residue is not encoded by *E. coli* tmRNA, but it is derived from that aminoacylated to tmRNA [13,15]. Thus, a model of *trans*-translation has been proposed: Ala-tmRNA enters the vacant A-site of the ribosome stalled at the 3′ end of a truncated mRNA, and it receives the nascent polypeptide from peptidyl-tRNA in the P-site, allowing resumption of translation with an exchange of the template from the truncated mRNA to the tag-encoding region on tmRNA [11]. Consequently, this process would facilitate ribosome recycling.

Accumulating genome sequence data have suggested that every bacterial cell is equipped with the *trans*-translation system mediated by tmRNA. The tRNA-like structure (TLD) shows high sequence conservation, while the other region of tmRNA is less conserved. Although the amino acid sequence of tag-peptide as well as its length is also less conserved, the sequence of the last four amino acids is highly conserved as AlaLeuAlaAla (ALAA). Since this sequence serves as the target for a periplasmic protease (Tsp) [11] and cytoplasmic ATP-dependent proteases (ClpXP, ClpAP, Lon and FtsH) [16–19], C-terminally truncated but tagged polypeptides, which might be mostly nonfunctional or sometimes deleterious, are preferentially degraded in the cell. Thus, the *trans*-translation system serves as a quality control system that prevents nonfunctional polypeptides produced from truncated mRNAs from accumulating in the cell. In addition, this system prevents truncated mRNAs themselves from accumulating in the cell, as described in a later subsection [20].

2.2. Mechanism of trans-translation

2.2.1. Structure of tmRNA and its binding proteins

Both terminal sequences of tmRNA form a tRNA-like structure with an amino acid acceptor stem, a deformed D-arm and a TΨC-arm with tRNA-specific modifications but with no apparent anticodon [8,9,21–23]. The third base pair position of the amino acid acceptor stem is occupied by a G-U non-Watson-Crick base pair, which serves as a potent recognition site for alanyl-tRNA synthetase. Typically, four pseudoknot structures forming a large ring are present in the middle of tmRNA, and the tag-encoding sequence is located between the first two pseudoknots (PK1 and PK2) [22,24–26]. The tRNA-like domain (TLD) and the pseudoknot-rich region are connected by a long helix with bulges and loops. The secondary structure of tmRNA is fairly well conserved, although it is separated into two pieces in some bacterial lineages [27].

In addition to tRNA processing enzymes [28] and alanyl-tRNA synthetase, three proteins, the ribosomal protein S1 [8], EF-Tu [29–31] and SmpB [32], can bind to tmRNA. S1 is not required for *trans*-translation reaction until the first peptidyl transfer [33,34], although it might have a role in a later process of *trans*-translation [35,36]. EF-Tu in complex with GTP (EF-Tu·GTP) binds to alanylated tmRNA (Ala-tmRNA) to deliver it to the ribosomal A-site as it does to aminoacyl-tRNA (aa-tRNA) in the elongation process of translation.

The most important binding partner of tmRNA for *trans*-translation is SmpB, which is absolutely conserved among bacteria as is tmRNA. Through binding to TLD of tmRNA [37–39], SmpB plays a role outside the ribosome in preventing tmRNA from degradation [38] and enhancing aminoacylation of tmRNA [38,40], and it is essential for the ribosomal processes of *trans*-translation [32,38]. SmpB has a globular core consisting of an antiparallel β-barrel with a C-terminal tail unstructured in solution [41,42]. Crystal structures

Table 1
Ribosome rescue factors in bacterial cells.

Main factor(s)	System	Product	Signal	Note	Refs.
tmRNA, SmpB, EF-Tu	*trans*-translation	Degradation	Non-stop mRNA	Ribosome recycling and other roles	[2,3,8,9,11,13]
ArfA, RF2		Truncated protein	Non-stop mRNA	Backup system for *trans*-translation	[4,5]
YaeJ (ArfB)		Truncated protein	Non-stop mRNA?	RF homologue	[6,7]
Pth	Drop-off	Truncated protein		At early stage of translation	[119–123]
EF-P		Complete protein	Consecutive Pro residues	tRNA mimic	[125–127]
EF4 (LepA)	Back translocation	Complete protein		At high Mg^{2+} ions or low temperature GTPase	[128,129]
HSP15, release factor?		Truncated protein	Dissociated ribosome	Heat shock protein	[130,131]

Please cite this article in press as: H. Himeno, et al., Ribosome rescue systems in bacteria, Biochimie (2014), http://dx.doi.org/10.1016/j.biochi.2014.11.014