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Insights into the ribosomal *trans*-translation rescue system: lessons from recent structural studies

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Running title: Structural snapshots of *trans*-translation

Keywords: cryo-EM, Ribosome, SmpB, tmRNA, *trans*-translation.

Abstract

The arrest of protein synthesis caused when ribosomes stall on an mRNA lacking a stop codon is a deadly risk for all cells. In bacteria, this situation is remedied by the *trans*-translation quality control system. *Trans*-translation occurs because of the synergistic action of two main partners, transfer-messenger RNA (tmRNA) and small protein B (SmpB). These act in complex to monitor protein synthesis, intervening when necessary to rescue stalled ribosomes. During this process, incomplete nascent peptides are tagged for destruction, problematic mRNAs are degraded, and the previously stalled ribosomes are recycled. In this “Structural Snapshot” article, we describe the mechanism at the molecular level, a view updated after the most recent structural studies using cryo-electron microscopy.

Introduction

Protein synthesis, or translation, is carried out by the ribonucleoprotein nanomachines called ribosomes. They read the information contained within messenger mRNA (mRNA) and catalyse polypeptide chains synthesis by forming peptide bonds between the amino-acids corresponding to each three-base triplet, or codon. To ensure that the genetic information is accurately translated, multiple quality control mechanisms are needed. One of the most critical outcomes of inefficient protein synthesis occurs when ribosomes stall along the mRNA molecule, something which notably occurs during translation of “non-stop” mRNA transcripts lacking a termination codon. The absence of a stop codon prevents translation termination, so ribosomes accumulate on the defective mRNA and form stalled polysomal chains that are no longer productive [1], thus undermining bacterial cell vitality. In addition, the translation of defective mRNA results in the production of truncated proteins which are potentially toxic for the cell. This phenomenon occurs frequently, for instance in *Escherichia coli* where ~2-4% of the translating ribosomes stall on a defective mRNA and need to be rescued [2]. To tackle the problem of “non-stop” stalling, all bacteria firstly use the same rescue system, *trans*-translation. This system is based on the synergy of transfer-messenger RNA (tmRNA), a chimeric molecule 260 to 420 nucleotides long (*e.g.* 363 nucleotides in *E. coli*) and its partner small protein B (SmpB) [3]. tmRNA is composed of a large pseudoknot ring (PK); a tRNA-like domain (TLD) and a messenger-like domain (MLD) that contains an internal open reading frame (ORF) devoid of a N-formylmethionine start codon (Fig. 1a). The tmRNA molecule begins by being transcribed as a non-mature precursor, which is then processed at its 5' and 3'-ends while nucleotides in the T-loop are modified at least twice [4–7]. Thanks to recognition of a G3:U base-pair as occurs in canonical tRNA^{Ala}, the TLD is always aminoacylated by alanine tRNA synthetase (AlaRS) [5]. The MLD encodes for the consensus sequence AN-[$-$]_n-ALAA (*e.g.*,

ANDENYALAA in *E. coli*) followed by a termination codon, allowing specific recognition of tagged proteins by proteases.

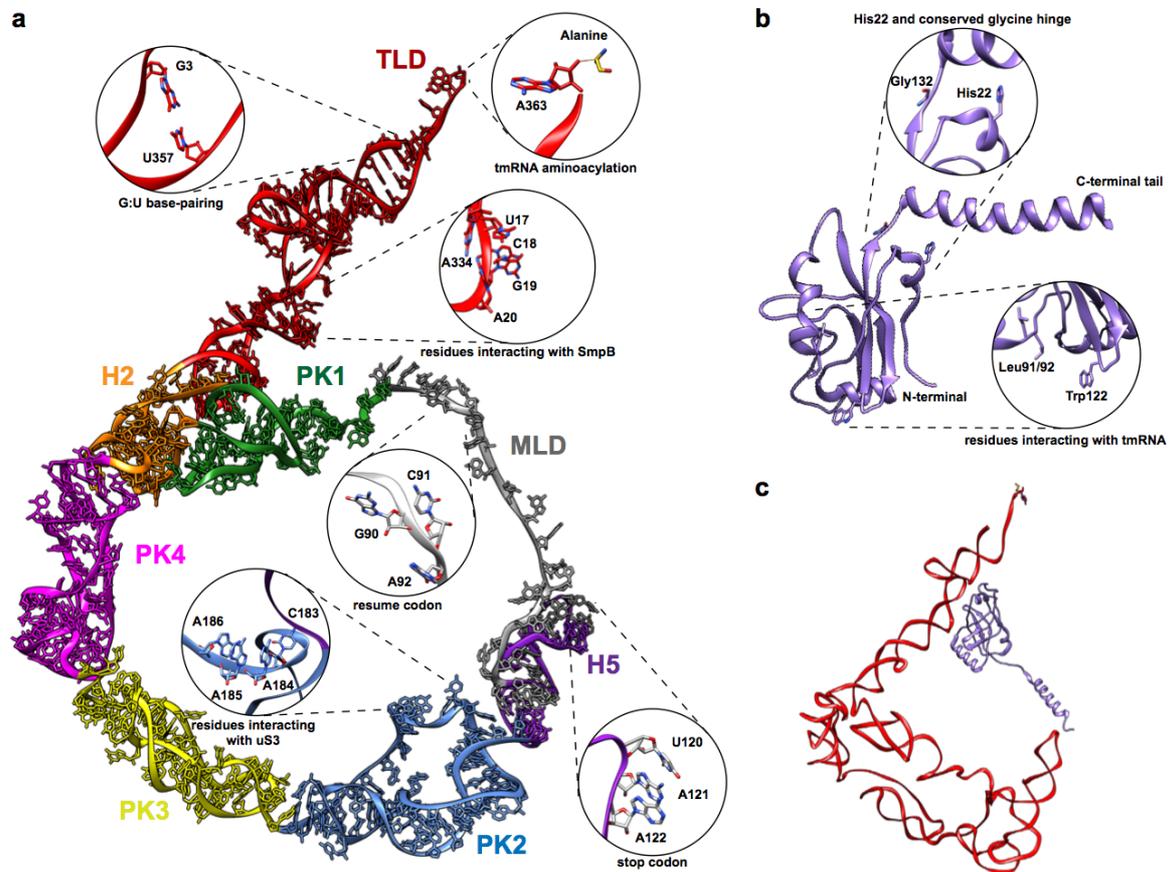


Figure 1: Elements in the *trans*-translation rescue system (a) 3D structure of a tmRNA molecule. The transfer-like domain (TLD) is red while helix H2 is orange. This is followed by the pseudoknot ring, made up of the four pseudoknots PK1 (dark green), PK2 (steel blue), PK3 (yellow), and PK4 (magenta). The MLD is grey, and the helix H5 is purple. The resume codon, stop codon, conserved G-U base pair, tmRNA aminoacylation, and the residues interacting with SmpB and uS3 are highlighted. (b) 3D structure of SmpB (purple). The residues interacting with the tmRNA, His22, and the conserved Gly132 are highlighted. (c) 3D structure of the tmRNA-SmpB complex when bound to the ribosome during unlocking of the stalled ribosome. SmpB (purple) binds to the TLD's D-loop in tmRNA (red).

The proteases involved in degrading tmRNA-tagged proteins are ClpXP/ClpAP, FtsH, and Tsp. During that process much, if not all, of the MLD's sequence conservation is important for recognition by cellular proteases and/or adaptor proteins [8]. The tmRNA partner SmpB is a small basic protein of ~160 amino acids. Its body is folded into an oligonucleotide/oligosaccharide-binding (OB) fold domain, while the last 30 residues form a tail unstructured in solution but which folds as an α -helix within the ribosome (Fig. 1b). SmpB

is tmRNA's most important associate, binding it with high affinity (~20 to 50nM) [9,10], in a molar ratio of 1:1 [11,12] (Fig. 1c). The other major actor in *trans*-translation is Ribonuclease R (RNase R), a 3'→5' exoribonuclease in the RNase II superfamily involved in degrading truncated non-stop mRNA [13]. During the rescue process (Fig. 2), the tmRNA TLD is bound to SmpB and accommodated into the vacant ribosomal A site. After transpeptidation occurs between the stalled incomplete peptide and the tmRNA alanine, a swap between the tmRNA messenger-like domain (MLD) and the original problematic mRNA allows translation to resume [14,15]. The ribosome translates the information on the tmRNA's ORF, just until it reaches the stop codon. The stalled ribosome is then recycled naturally into free subunits, while the incomplete peptides are tagged with a signal sequence that results in proteolysis. The problematic mRNAs meet the same fate, as they are degraded by RNase R (for a recent complete review, see reference [3]).

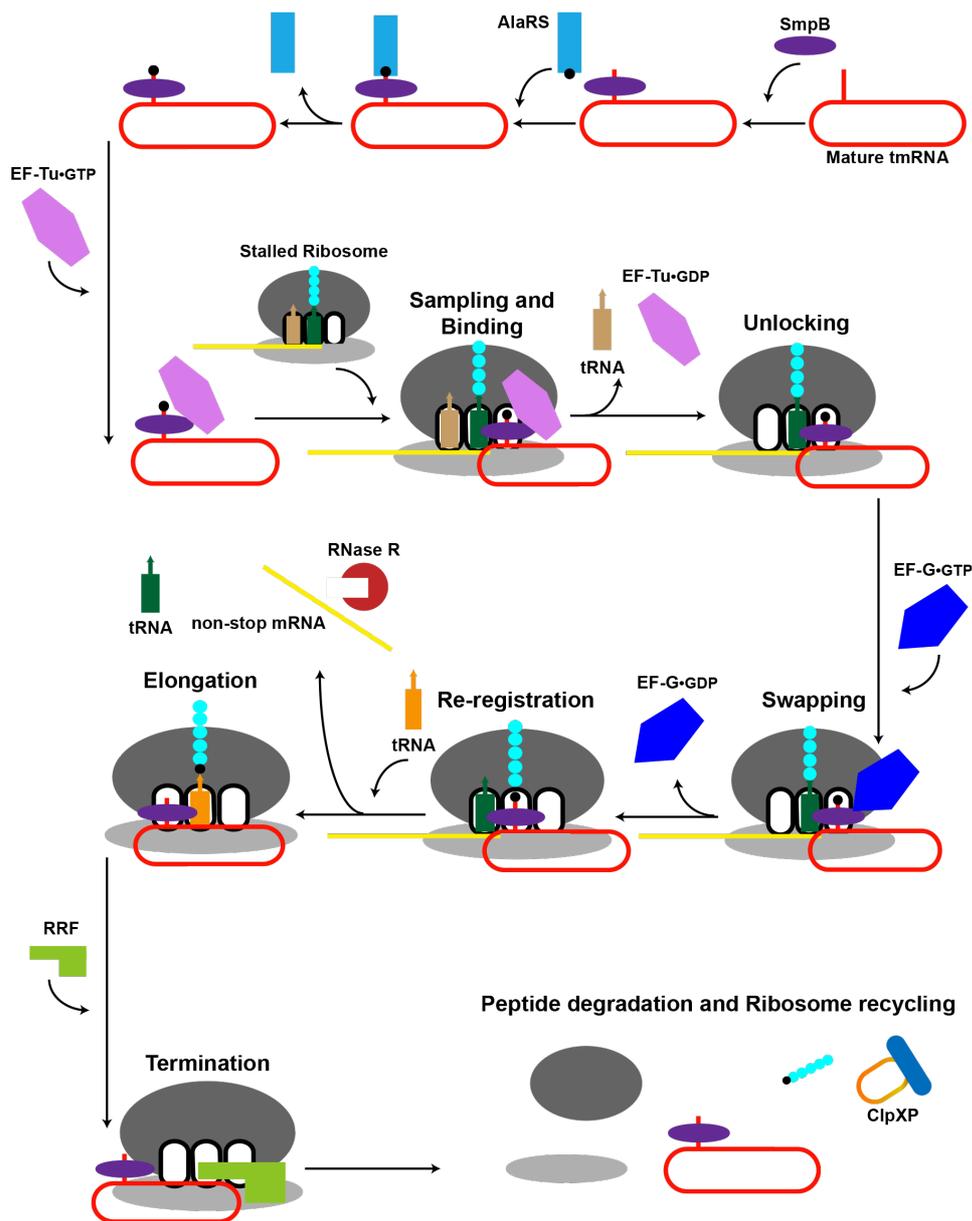


Figure 2: The complete *trans*-translation quality control cycle.

Ribosome sampling, binding and unlocking: Mature tmRNA associates with its partner SmpB to form a complex. AlaRS charges the complex with an alanine, and the enzyme is released. EF-Tu•GTP binds to tmRNA-SmpB to form the quaternary complex needed to rescue the ribosome stalled on a non-stop mRNA. To recognize these ribosomes the quaternary complex enters the ribosomal A site. There, SmpB mimics a codon-anticodon pairing while its C-terminal tail is inserted in the mRNA channel. If the tail fits in the empty mRNA channel the elongation factor EF-Tu•GDP is released after GTP hydrolysis. The Ala-tmRNA-SmpB complex is then accommodated into the A site, triggering the peptidyl transfer reaction. **mRNA swapping and re-registration:** EF-G•GTP helps shift the tmRNA-SmpB into the P site thanks to hydrolysis of its GTP. EF-G•GDP is released, and the non-stop mRNA is ejected and then degraded by RNase R. The tmRNA's ORF is placed into the A site and new tRNAs arrive at the ribosome to resume translation. **Elongation, termination, peptide degradation and ribosome recycling:** The tmRNA-SmpB complex moves towards the E site and the TLD and SmpB are promptly ejected, while the translation of the MLD continues until the ribosome reaches the MLD's stop codon. The ribosome subunits are then dissociated by the Ribosome Recycling Factor RRF and the nascent peptide is degraded by ClpXP/ClpAP/FtsH/Tsp proteases. All of the components are recycled and ready for a new round.

Remarkably, *trans*-translation is present in nearly all bacteria [16], and it is their main mechanism to start afresh, preventing cell death. Some bacteria also have back-up rescue processes based on the alternative ribosome-rescue factors A and B (ArfA and ArfB) [17,18] or using ribosome-associated quality control (RQC) pathways mediated by RqcH and RqcP [3,19]. Recent studies have also reported alternative systems based on ArfT in *Francisella tularensis* and on BrfA in *Bacillus subtilis* [20,21]. However, these backup mechanisms are imperfect as none of them is able to accomplish with specificity the three outcomes of *trans*-translation, *i.e* not only ribosome recycling but also protein and RNA targeted degradation. This is likely why *trans*-translation predominates and why, if some bacteria use only *trans*-translation, none use only ArfA or ArfB [22]. Therefore, and despite their physiological importance [18,23], one can expect that even when these are present, they are not enough to ensure steady and prolonged cell fitness, particularly in the presence of stress, this highlights the great importance of *trans*-translation for bacterial survival.

Based on the most recent data produced by cryo-electron microscopy (cryo-EM) [24,25], we focus here on the structural foundation for the perfect synchronicity seen between the various partners of *trans*-translation.

The carefully choreographed ballet of *trans*-translation

We can divide *trans*-translation into different stages based on both the similarities and differences between it and canonical translation. The first “ribosome sampling, binding and unlocking” step covers the identification of stalled ribosomes and transpeptidation of the stalled incomplete polypeptide on the tmRNA TLD. Next, in “mRNA swapping and re-registration”, the MLD elongates and problematic mRNA is degraded. Finally, in “peptide degradation and ribosome recycling”, tagged peptides are released and degraded, and the ribosomes are recycled.

1. Ribosome sampling, binding, and unlocking

We can further separate this step into two consecutive states related to canonical translation: pre-accommodation and accommodation. During pre-accommodation, the quaternary complex (tmRNA–SmpB–EF-Tu•GTP) recognizes and enters the stalled ribosome [26]. This process involves several coordinated actions (Fig. 3). The acceptor and T-arms of tmRNA's alanylated TLD are bound to EF-Tu in its GTP state, while the TLD elbow region formed by the D and variable loops is in close contact with the body of SmpB.

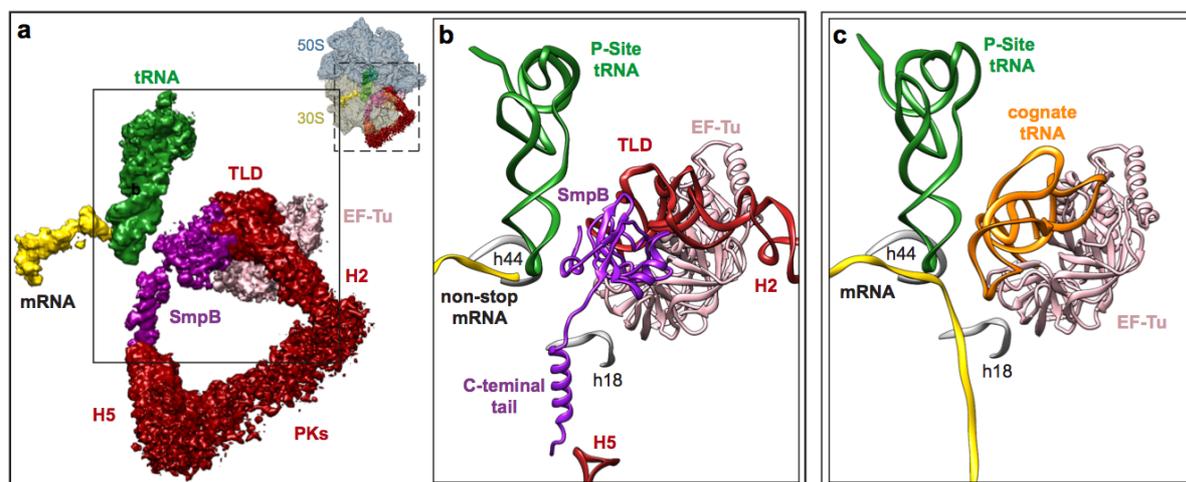


Figure 3. Highlight on the interactions between the main *trans*-translation actors and the stalled ribosome during the initial binding step (a) Overview of the *trans*-translating ribosome and focus on the characteristic spatial arrangements of EF-Tu, tmRNA, SmpB, P-Site tRNA, and non-stop mRNA (PDB 7ABZ, EMDB EMD-11710). The 50S subunit is light blue, the 30S is gray, EF-Tu is pink, tmRNA is red, SmpB is magenta, the P-Site tRNA is green, and non-stop mRNA is yellow. The map is contoured at 2σ and only for the regions within 2\AA of the non-stop mRNA, P-site tRNA, tmRNA and SmpB. **(b)** Details of the interactions between the tmRNA tRNA-like domain (TLD), the H5 helix, SmpB and EF-Tu. The P-Site tRNA, mRNA, and the decoding center (16S rRNA helices h18 and h44) are also shown. **(c)** Comparison with the pre-accommodation state of a cognate tRNA (orange) in the 30S closed conformation (PDB 6WD9, EMDB EMD-21628) with a long mRNA (modelled from PDB 6BY1). The TLD mimics the Acceptor- and T-arms of tRNA while the core of SmpB mimics the anticodon stem of tRNA during initial codon selection. SmpB long C-terminal tail folds in the mRNA channel whose entrance is closed by the tip of helix H5. This insures that the complex does not interfere with canonical translation.

Stalled ribosomes are selected by SmpB, which uses its C-terminal tail to probe the occupancy of the ribosomal mRNA channel inside and downstream from the decoding site (Fig. 3a-b). If the channel is already occupied by an mRNA, SmpB cannot insert its tail, and this is how *trans*-

translation avoids interfering with canonical translation (Fig. 3c). On the other hand, if it is available, the C-terminal tail extends along the mRNA path and forms an α -helix whose extremity is in contact with the tmRNA helix H5. This specific interaction is surely instrumental for the complete recognition of the empty A site and downstream mRNA channel which would otherwise be impossible in the presence of a full-length mRNA. Additionally, the binding of PK2, which anchors H5 at the entrance to the mRNA channel, seems to be the more important interaction for positioning of the MLD into the channel. During this step, SmpB mimics a codon-anticodon interaction, as if a correct tRNA-mRNA interaction was occurring. Once on the ribosome, EF-Tu contacts the 50S subunit, further stabilizing the entire complex. The 30S subunit is in a closed conformation that resembles that of the equivalent ternary complex tRNA-EF-Tu•GTP [12,27,28]. The L-shaped TLD forms a 120°-angle, while the CCA 3'-end of the acceptor stem is in the same conformation as observed in canonical tRNA [12,25,29,30]. Just upstream from the TLD acceptor stem, the helix H2 lies between the two ribosomal subunits, allowing the MLD, helix H5, and the pseudoknots to form a “PK ring” surrounding the 30S “beak” [24–26,31,32]. At this point, the only contact between the PK ring and the ribosome occurs through PK2 and H5: PK2 is in close contact with the uS3 RNA-binding domain, while helix H5 crowds the groove formed by uS3, uS4 and uS5 at the entrance of the mRNA channel. Inside the Ribosome decoding center, the 16S nucleotide A1492 remains within helix h44, while A1493 is partially flipped out toward the major groove because of its interaction with the SmpB well-conserved residue His22 (Fig. 4 a-c). This conformation is different from that of canonical decoding in which the binding of mRNA and cognate tRNA in the A site induces A1492 and A1493 to flip out of the internal loop of h44 and interact, respectively, with the first and second base pairs of the codon-anticodon helix (Fig. 4 d-e) [33].

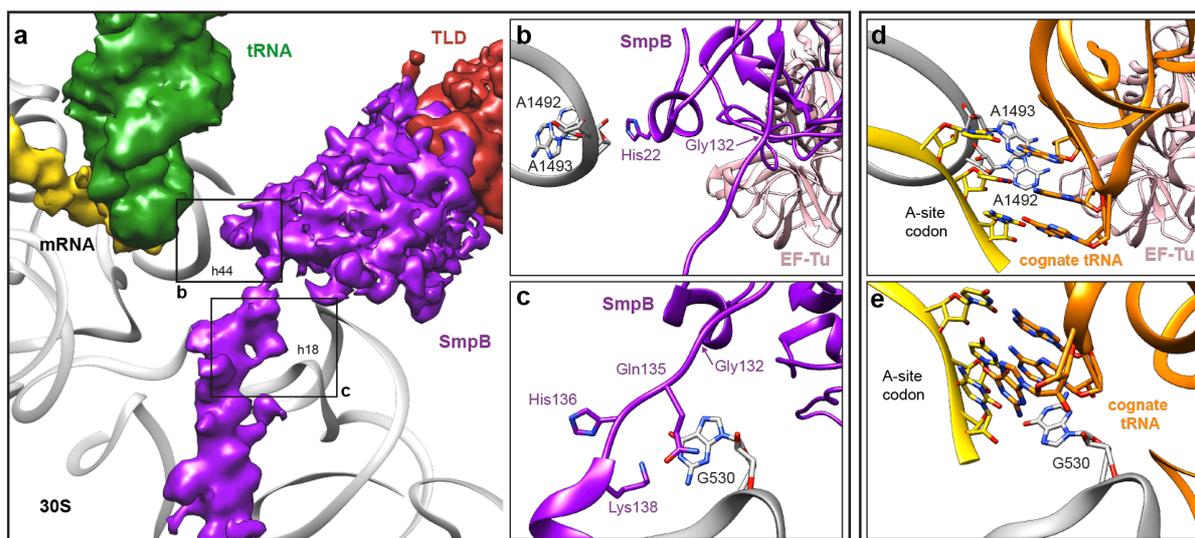


Figure 4. Interaction of SmpB with the decoding center (DC) during the binding step. (a) Overview of the DC during the binding of the tmRNA-SmpB-EF-Tu•GTP quaternary complex to the stalled ribosome (PDB 7ABZ, EMDB EMD-11710). (b) Close-up view of the interactions between the first α -helix of SmpB and nucleotides A1492 and A1493 of 16S rRNA. (c) Close-up view of the interactions between SmpB C-terminal tail and G530 of 16S rRNA. Residues and nucleotides within 4 Å of each other are indicated. The position of the conserved Gly132 is also indicated. (d) Comparison with interactions observed between mRNA, cognate tRNA, and nucleotides A1492 and A1493 of 16S rRNA during the pre-accommodation state of a cognate tRNA (PDB 6WD9, EMDB EMD-21628). (e) Same as (d) but with the nucleotide G530. The 16S rRNA is gray, SmpB is magenta, tRNA is green, and non-stop mRNA is yellow. The map is contoured at 2σ and only for the regions within 2 Å of the non-stop mRNA, P-site tRNA, tmRNA and SmpB.

As observed during canonical translation, the B1a bridge is open, and the B1b/c bridges are closed [34–36]. This pre-accommodation state favours GTP hydrolysis in EF-Tu as well as conformational changes in the ribosomal subunits, inducing accommodation (Fig. 5).

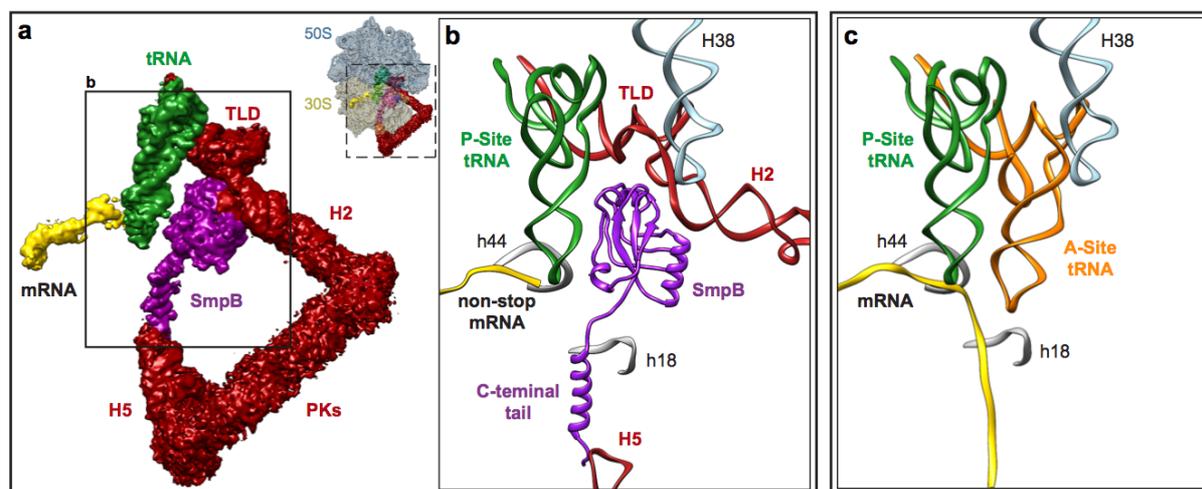


Figure 5. Highlight on the interactions between the main *trans*-translation actors and the stalled ribosome during the unlocking step (a) Overview of the *trans*-translating ribosome and focus on the characteristic spatial arrangements of tmRNA, SmpB, P-Site tRNA, and non-stop mRNA inside the ribosome

(PDB 7AC7, EMDB EMD-11713). The 50S subunit is light blue, the 30S is gray, tmRNA is red, SmpB is magenta, the P-Site tRNA is green, and non-stop mRNA is yellow. The map is contoured at 2σ and only for the regions within 2\AA of the non-stop mRNA, P-site tRNA, tmRNA and SmpB. **(b)** Details of the interactions between the tmRNA tRNA-like domain (TLD), the H5 helix, SmpB, and the P-Site tRNA. The mRNA, 23S rRNA helix H38, and 16S rRNA helices h18 and h44 are also shown. **(c)** Comparison with the structure of a cognate tRNA (orange) accommodated in the A site (PDB 7K00, EMDB EMD-22586) in the presence of a long non-truncated mRNA (modelled from PDB 6BY1). The TLD perfectly mimics the Acceptor- and T-arms of A-Site tRNA within the peptidyl-transferase center (PTC). The core of SmpB rotates to replace the codon-anticodon interactions observed during canonical translation. SmpB long C-terminal tail still occupies the mRNA channel and its interactions with helix H5 are reinforced before re-registration starts.

The EF-Tu•GDP complex is then released from the ribosome, and SmpB moves more deeply into the A site. As compared with pre-accommodation, the PK ring is now larger and the MLD portion is unfolded – with the exception of a hairpin between U88 and A100 whose presence was earlier confirmed by biochemical assays [37] – meaning that it is ready to enter the mRNA path. The TLD region switches into the peptidyl transferase centre (PTC), accommodated in the same way as correct tRNA [38]. The TLD's T-loop interacts with both uL16 and 23S rRNA helices H38 and H89, while the D-loop is still in contact with SmpB although the protein rotated by $\sim 30^\circ$. The SmpB C-terminal tail remains folded into a helix within the mRNA channel. In the decoding centre, A1493 is now completely outside and forms π -stacking with H22, while its partner A1492 is in an intermediate conformation, partially flipped out of helix 44 (Fig. 6) in a scenario that resembles a ribosome with a vacant A site during canonical translation [39].

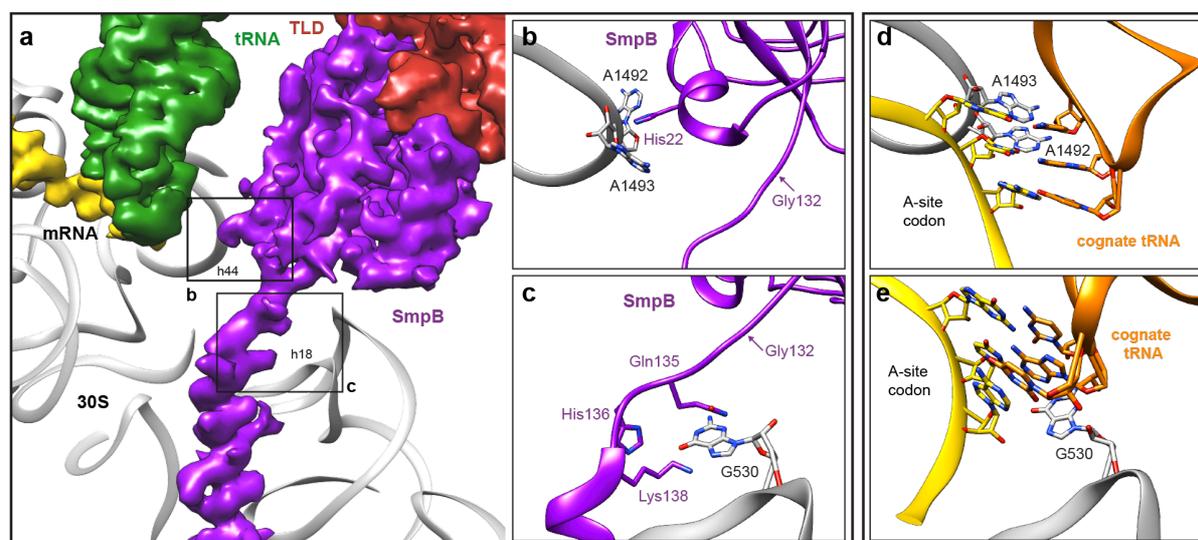


Figure 6. Interaction of SmpB with the decoding center (DC) during the unlocking step.

(a) Overview of the DC during the binding of the tmRNA-SmpB-EF-Tu•GTP quaternary complex to the stalled ribosome (PDB 7AC7, EMDB EMD-11713). **(b)** Close-up view of the interactions between the first α -helix of SmpB and nucleotides A1492 and A1493 of 16S rRNA. **(c)** Close-up view of the interactions between SmpB C-terminal tail and G530 of 16S rRNA. Residues and nucleotides within 4 Å of each other are indicated. The position of the conserved Gly132 is also indicated. **(d)** Comparison with interactions observed between mRNA, cognate tRNA, and nucleotides A1492 and A1493 of 16S rRNA a translating ribosome with tRNAs in the A and P sites (PDB 7K00, EMDB EMD-22586). **(e)** Same as (d) but with the nucleotide G530. The 16S rRNA is gray, SmpB is magenta, tRNA is green, and non-stop mRNA is yellow. The map is contoured at 2σ and only for the regions within 2Å of the non-stop mRNA, P-site tRNA, tmRNA and SmpB.

The interaction between PK2 and uS3 is maintained, while helix H5 blocks the mRNA entrance channel completely, in close contact with uS3, uS4, uS5, and the extremity of the SmpB C-terminal tail. Unlocking then occurs once the truncated peptide is displaced from the P-site tRNA to the tmRNA alanine during transpeptidation. This reaction is accompanied by a partial rotation of the 30S head relative to the 50S subunit [24,25,40]: the small and large subunits rotate by 8-10° with respect to each other while the head undergoes a simultaneous rotation of 5-7° with respect to the body [41,42]. These movements induce a shift of both tmRNA and tRNA into intermediate A/P and P/E binding states, respectively. EF-G then enters the ribosome, tmRNA is translocated into the P site, and the stalled tRNA passes through the E site and is ejected from the ribosome (Fig. 7).

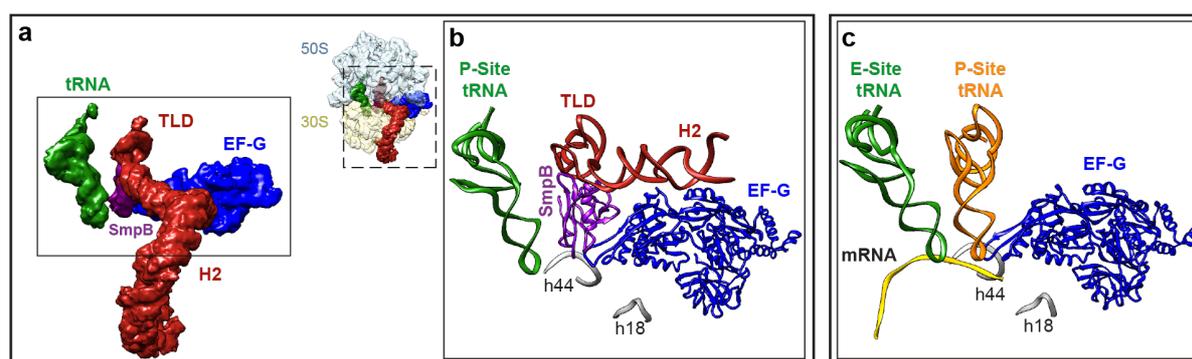


Figure 7. Highlight on the interactions between the main *trans*-translation actors and the ribosome during the swapping (a) Overview of the *trans*-translating ribosome and focus on the characteristic spatial arrangements of tmRNA, SmpB, E-Site tRNA and EF-G inside the ribosome (PDB 4V6T, EMDB EMD-5386). The 50S subunit is light blue, the 30S is gray, tmRNA is red, SmpB is magenta, the E-Site tRNA is green, EF-G is blue. (b) Details of the interactions between the tmRNA tRNA-like domain (TLD), SmpB, EF-G, and the E-Site tRNA. 16S rRNA helices h18 and h44 are also shown. *Note that the map resolution did not permit to build an atomic model of SmpB C-terminal tail.* (c) Comparison with the structure of a P-site tRNA (orange) (PDB 7K52, EMDB: EMD-22671).

Here, the presence of EF-G is associated with classical large-scale conformational changes of the small 30S subunit (ref [43]), including a $\sim 5^\circ$ rotation of the body/platform domains while the head is swivelled by an additional $\sim 19^\circ$. However, during *trans*-translation, this step is characterized by a unique additional 12° tilt movement around an axis that is approximately parallel to the mRNA, around the A- and P-site codons. These rotational head movements link TLD translocation to MLD loading, showcasing how the elements move within the ribosome [44]. B1a and B1b bridges between the two ribosomal subunits open, thereby allowing the tmRNA helix H2 to slide between these subunits (see below). After GTP hydrolysis, the EF-G•GDP complex dissociates from the ribosome, and the subunits return to their initial state.

As the release of non-stop ribosomes is essential for cell survival, and the main rescue system for this is *trans*-translation, it has to be noted that this process is a very appealing target in the development of new antimicrobial compounds [45,46], especially since it is absent in eukaryotes [47]. Impairing any of the specific interactions that occur between SmpB, tmRNA and the ribosome is therefore a promising strategy for killing pathogenic bacteria or for making them extremely sensitive to existing antibiotics. For instance, a recent cryo-EM structural study performed in the absence of a tmRNA-SmpB complex suggests that the acylaminooxadiazoles bind ribosomes near the peptidyl transferase centre, in a position that would clash with tmRNA in the A site, thereby inhibiting *trans*-translation by preventing tmRNA-SmpB binding described here, or else by interfering with the translocation of the complex from the A to the P site [48]. Moreover, it changes the conformation of ribosomal protein bL27 which, in this position would not be able to participate in the peptidyl transferase reaction when tmRNA is presented in the A site.

2. mRNA swapping and re-registration

While the TLD-SmpB complex is transferred to the P site in a translocation state related to canonical translation, the problematic non-stop mRNA and the MLD are swapped. In this new state, the PK ring completely changes its conformation so that the MLD can enter the mRNA channel in an unusual initiation mechanism (Fig. 8).

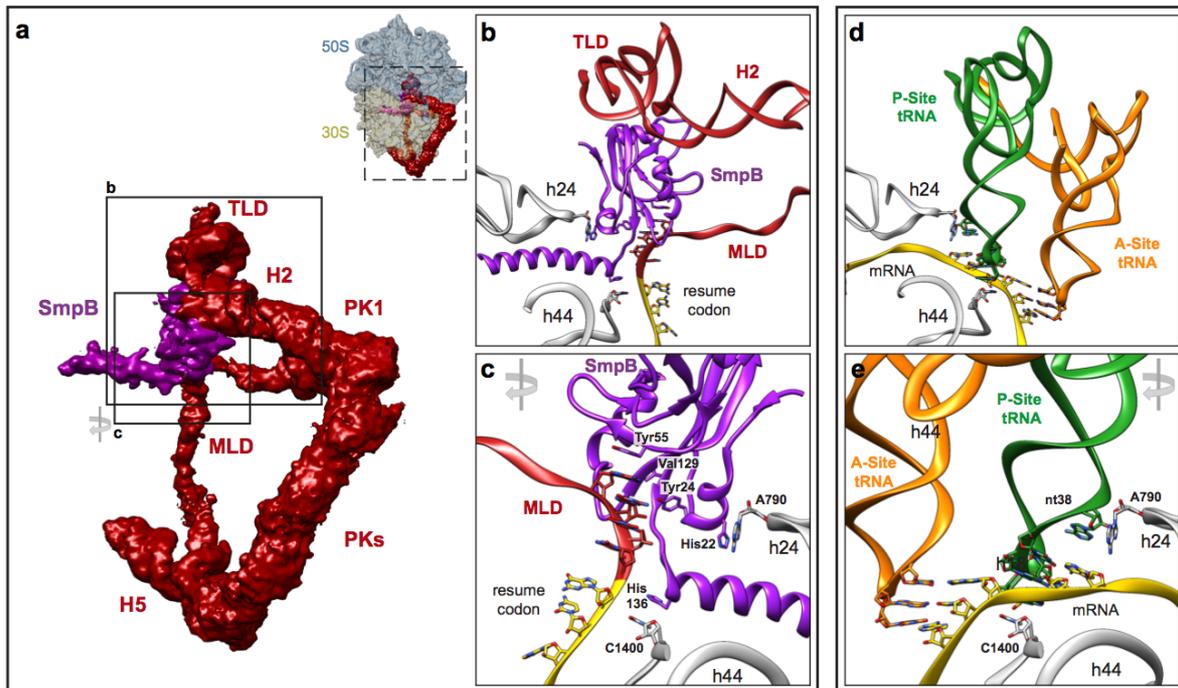


Figure 8. Highlight on the interactions between the main *trans*-translation actors and the ribosome during the re-registration step (a) Overview of the *trans*-translating ribosome and focus on the characteristic spatial arrangements of tmRNA and SmpB inside the ribosome (PDB 7ACJ, EMD-11717). The 50S subunit is light blue, the 30S is gray, tmRNA is red and SmpB is magenta. The map is contoured at 2σ and only for the regions within 2\AA of tmRNA and SmpB. (b) Details of the interactions between tRNA like domain (TLD) and messenger-like domain (MLD) of tmRNA and SmpB and the mRNA exit channel (16S helices h24 and h44). The position of the resume codon (yellow) is highlighted. (c) Close-up view on the interactions between SmpB Tyr24, Tyr55 and Val129, and the four nucleotides upstream the resume codon. The view is rotated by 180° . (d-e) Comparison with structure of a translating ribosome (PDB 7K00, EMD EMD-22586) with a long non-truncated mRNA (modelled from PDB 6BY1). The SmpB C-terminal tail occupies the mRNA exit channel and its globular body stacks with A790 and C1400 of 16 rRNA, just like a P-Site tRNA. As a result, the resume codon is correctly placed into the vacant A site.

To do this, the MLD must first pass through the 30S latch (or A-site latch), a physical barrier present between the head (helix 34) and body (guanosine 530) of the 16S rRNA when the small subunit is in a closed conformation [24,44]. Successful loading of the MLD into the path occurs when bridges B1a and B1b open, which is only possible after the 30S head undergoes a rotation

of 21° and a tilt of 3° [44]. Helix H5 moves away from uS4 and uS5 to free up the mRNA channel and to permit the MLD to enter. MLD loading is accomplished during the backward movements repositioning the 30S head: by staying associated with S3, the MLD is directly guided through the opened latch into the mRNA path [44]. The nucleotides forming the tip of the helix are stabilized by uS2 while the beginning of the helix and the end of the ORF interact with uS3, uS4 and uS5. SmpB's body is still interacting strongly with the TLD, but the C-terminal tail goes through an important conformational change during this translocation step. Indeed, in order for the MLD to enter the mRNA channel and the A-site, the tail of the protein must liberate the space it had previously occupied. This portion of the protein therefore undergoes a large 62° rotation, flipping over to the other side of the mRNA path within the E site. These data confirm that the highly conserved Glycine 132 acts as a flexible hinge that enables movement of the C-terminal tail, thus permitting proper positioning and establishment of the tmRNA open reading frame (ORF) as the surrogate template [49]. The non-stop mRNA is then released and degraded by RNase R. Re-registration occurs when the resume codon is precisely positioned into the decoding centre. This is made possible by direct interactions between SmpB Tyr24, Tyr55 and Val129 and the four nucleotides just upstream from the MLD resume codon (Figure 8), thereby ensuring greater control of the sequence and limiting the frameshift risks [50–53]. Translation then re-registers on the tmRNA's internal ORF, and a new aminoacyl-tRNA complex binds to the resume codon.

3. Peptide degradation and ribosome recycling

Based on recent structural studies and our knowledge in the field of canonical translation, it is pretty clear how translation of the entire MLD must continue. The tmRNA-SmpB complex should shift towards the E site and then leave the ribosome in such a way that the truncated peptide is transferred on the next tRNAs that are encoded by the following ORF codons, right

up until the stop codon is reached. Surprisingly, there have been no reports of tmRNA-SmpB in the E site, and instead the complex seems to move past that site to arrive directly at the solvent side of the ribosome [24]. Indeed, the risk of clashing with the ribosome in the E site makes it unlikely that the complex would remain stable in this step. After the post-E site move the MLD will then have to stretch along the mRNA path [54,55] and the H5 helix will have to unfold, certainly helped by the helicase properties of the ribosomal proteins uS2, uS4, and uS5. Indeed, these interactions are certainly linked to the helicase activity of these ribosomal proteins, already observed in the presence of structured mRNA molecules [56–59]. Although the later steps of *trans*-translation, *i.e.* translation and termination, have not yet been visualized in atomic detail, they are presumed to be canonical. Once the stop codon reaches the decoding centre, RF1 or RF2 release factors enter into the A site, freeing the tagged peptide so it can then be degraded by proteases. However, the release of tmRNA from the 30S could be more complicated due to its large structure wrapping around the 30S. The rescue process ends with the recycling of the ribosome (Fig. 9 and Movie 1).

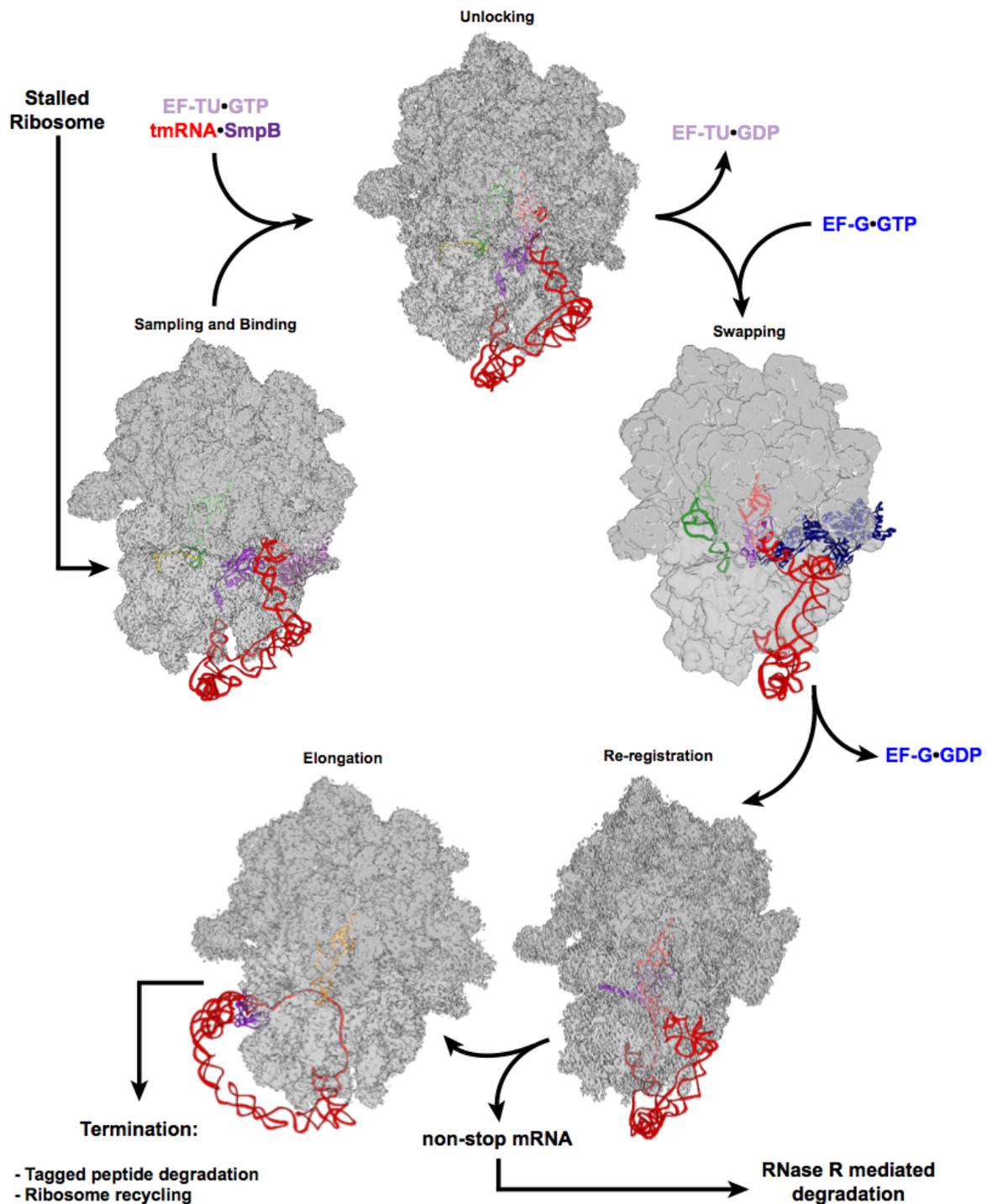


Figure 9: Structural overview of *trans*-translation. The steps structurally described are: **the non-stop stalled ribosome binding** (PDB 7ABZ, EMD EMD-11710); **unlocking** (PDB 7AC7, EMD-11713); **swapping** (PDB 4V6T, EMD-5386); **re-registration** (PDB 7ACJ, EMD-11717); and **elongation** (PDB 6Q9A, EMD-4478). tmRNA is red, SmpB is purple, P-site tRNA is green, non-stop mRNA is yellow, EF-Tu is pink, EF-G is blue, and the ribosome is gray.

Conclusions

The recent high-resolution cryo-EM structures have yielded a better understanding of how *trans*-translation occurs, revealing why this fascinating process does not interfere with canonical translation but also how it tricks the ribosome into continuing its catalytic activities on such a highly unusual substrate. Ten years ago, we still thought of SmpB as the simple “*handyman of tmRNA*” [60]. However, the studies showcase the crucial role played by SmpB during each and every step of tmRNA quality control. Indeed, its work begins early and outside the ribosome, when it binds to the TLD of tmRNA, protecting it from degradation [9] and enhancing the efficiency of alanylation [10,61,62]. It is then not just instrumental but in fact mandatory to tmRNA for recognizing and binding stalled ribosomes. To achieve this, SmpB mimics a codon-anticodon pairing within the vacant ribosomal A site and extends its C-terminal tail downstream into the mRNA channel, thereby establishing specific interactions with the ribosome. In the next step of translocation, the protein goes through an important conformational change, mimicking the interactions of a tRNA anticodon loop in the P site. This allows for the correct positioning of the MLD resume codon as well as the ejection of non-stop mRNA. SmpB continues to accompany tmRNA during the subsequent rounds of translation of the MLD, protecting tmRNA as its TLD emerges from the ribosome [11]. In fact, SmpB is more like a “*conductor*” of the *trans*-translation symphony orchestra organized to overcome stalled ribosomes, as it is involved throughout the entire quality control process.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

GU and RG wrote the first draft of the manuscript. All authors participated in manuscript revision and approved its final version.

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