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Trans-Translation: The tmRNA-Mediated Surveillance Mechanism for Ribosome Rescue, Directed Protein Degradation, and Nonstop mRNA Decay†

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ABSTRACT: The accurate flow of genetic information from DNA to RNA to protein is essential for all living organisms. An astonishing array of quality-assurance mechanisms have evolved to ensure that high degree of fidelity is maintained at every stage of this process. One of the most fascinating quality-control mechanisms involves tmRNA, also known as SsrA or 10Sa RNA. tmRNA is a versatile and highly conserved bacterial molecule endowed with the combined structural and functional properties of both a tRNA and a mRNA. The tmRNA system orchestrates three key biological functions: (1) recognition and rescue of ribosomes stalled on aberrant mRNAs, (2) disposal of the causative defective mRNAs, and (3) addition of a degradation tag to ribosome-associated protein fragments for directed proteolysis. Although not essential in *Escherichia coli*, tmRNA activity is essential for bacterial survival under adverse conditions and for virulence in some, and perhaps all, pathogenic bacteria. Recent evidence suggests that in addition to its quality-control function the tmRNA system might also play a key regulatory role in certain physiological pathways. This review will focus on recent advances in our understanding of the structural properties, mechanistic details, and physiological significance of this unique RNA and its principal protein partners.

Messenger RNAs normally contain all the necessary information required for successful synthesis of proteins. In bacteria, the signals required for recognition by ribosomes, the Shine−Delgarno sequence and the AUG start codon, are located near the 5′ end of the mRNA.[1] Multiple ribosomes successively read each message, and each ribosome decodes consecutive codons in a 5′ to 3′ direction until a stop codon is reached. Stop codons not only signal the end of the protein coding sequence but also serve as the binding site for release factors, which promote release of the nascent polypeptide and facilitate recycling of ribosomes for further rounds of translation. Messenger RNAs that lack appropriate termination signals cause the accumulation of stalled ribosomes (*1*−*3*), as these mRNAs are unable to promote release factor

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[1] Abbreviations: tRNA, transfer RNA; mRNA, messenger RNA; tmRNA, transfer−messenger RNA; SsrA, small stable RNA A; ORF, open reading frame; nonstop mRNA, mRNA lacking a STOP codon; poly-U, polyuridine; PK, pseudoknot; D-loop, dihydrouridine loop; SmpB, small protein B; RNase, ribonuclease; A-site, aminoacyl tRNA binding site; P-site, peptidyl tRNA binding site; EF-Tu, elongation factor Tu; Ala-RS, alanyl-tRNA synthetase; GTP, guanosine triphosphate; GTPase, guanosine triphosphatase; IL-6, interleukin-6; NMR, nuclear magnetic resonance; cryo-EM, cryo-electron microscopy; Yops, *Yersinia* outer proteins.

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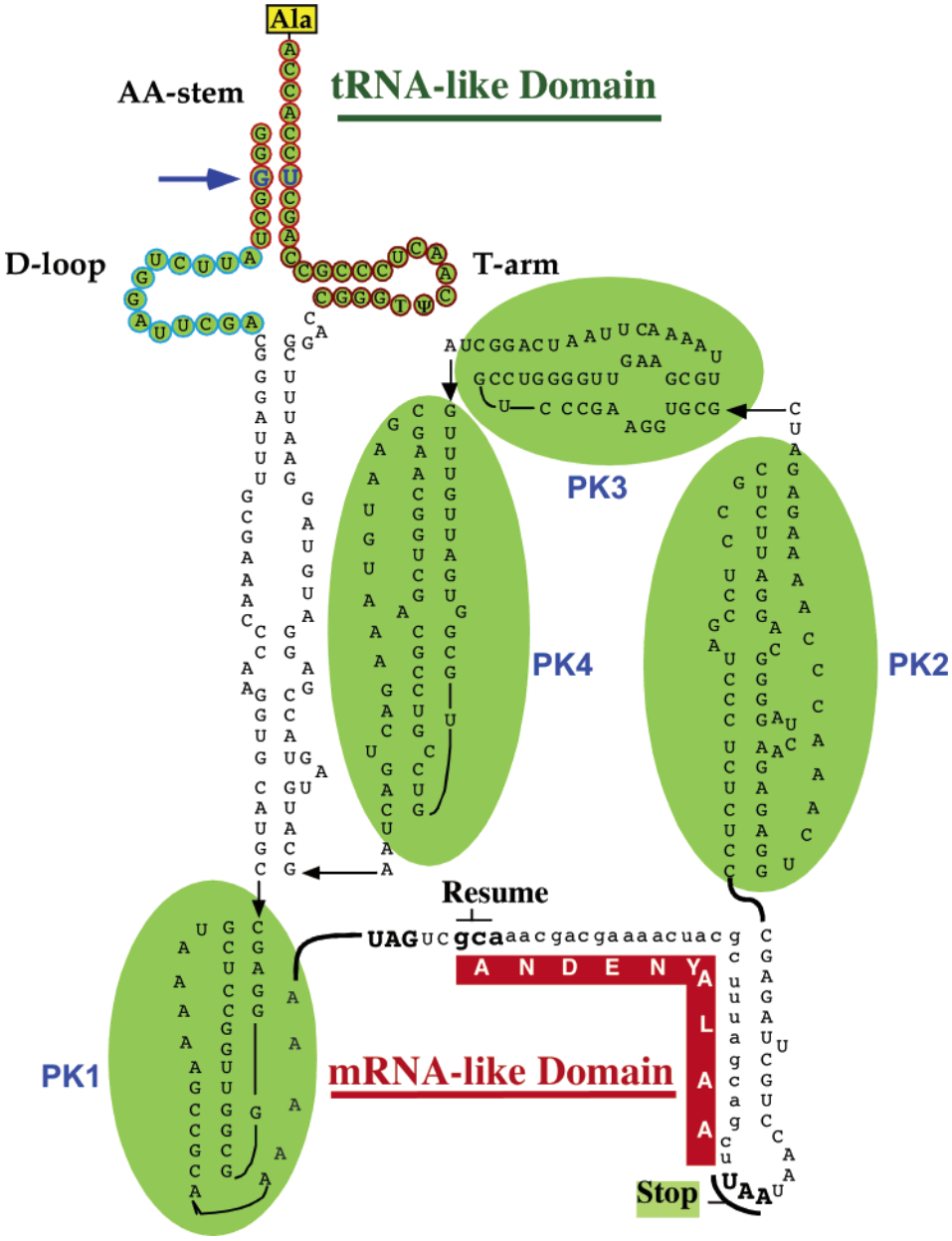

FIGURE 1: Proposed secondary structure of *E. coli* tmRNA. The tRNA- and mRNA-like properties are highlighted, including the amino acid acceptor stem (AA-stem, with a G·U wobble base pair), the D-loop, and the T-arm. The mRNA-like domain, with the upstream conserved UAG and the GCA resume signals, the tag peptide coding sequence, and the UAA termination signal are highlighted. The four pseudoknots are labeled PK1−4.

binding, nascent polypeptide release, and ribosome recycling. Accumulation of unproductively stalled ribosomes leads to three undesirable consequences for the cell. First, defective mRNAs cause a general and significant loss of translational efficiency, due largely to sequesteration and unavailablity of ribosomes for translation of new mRNAs. Second, the aberrent mRNAs, if not promptly removed, could engage the ribosomal machinery in additional futile cycles of translation. Third, the aberrant protein products translated from defective mRNAs, if released, might be deleterious for the cell.

How do bacteria cope with problems posed by defective mRNAs? Bacteria have evolved a unique translation quality-control system, comprised of SmpB protein and tmRNA, to solve the three challenges associated with aberrant mRNAs. This sytem recognizes and rescues stalled ribosomes, enables the decay of the causative aberrant mRNAs, and directs the addition of a C-terminal proteolysis tag to incomplete protein products.

Structure and Function of the tmRNA System Components

tmRNA Structure and Functional Domains. The predicted secondary structure of tmRNA (*1*, *4*, *5*) is depicted in Figure 1. The 5′ and 3′ ends of the RNA form a tRNA-like domain, including an acceptor stem, a T-arm, and a D-loop with no stem. Instead of an anticodon stem−loop structure, a long disrupted stem links the tRNA-like domain to the rest of the tmRNA molecule. A pseudoknot (PK1) connects the tRNA-like domain to the peptide reading frame, which ends with a stop codon in a hairpin loop. Three additional pseudoknots (PK2−4) link the mRNA-like domain to the 3′ end of the RNA.

The tRNA-like properties of the folded 5′ and 3′ ends of the molecule were first proposed and subsequently demon-

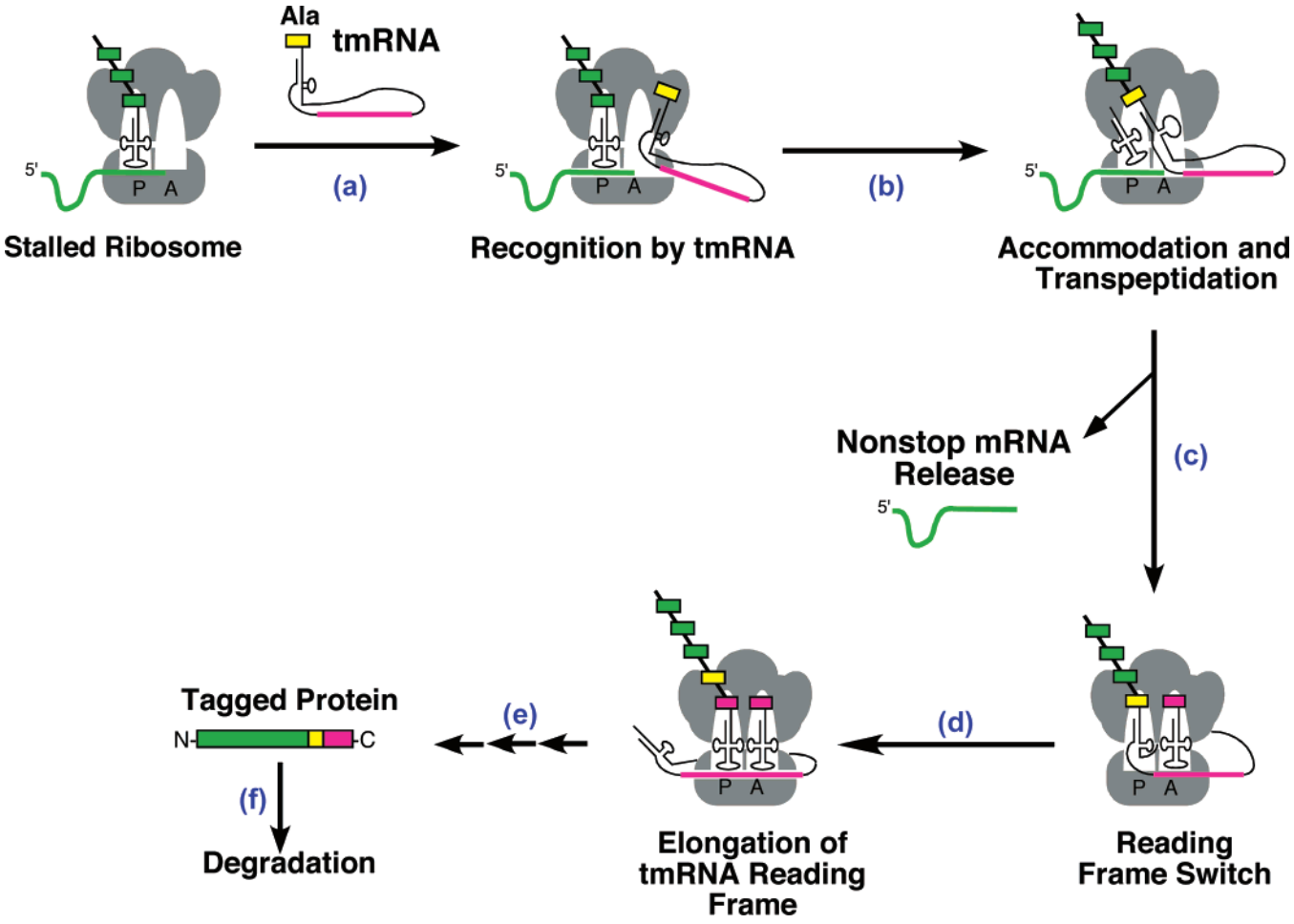

FIGURE 2: *Trans*-translation model for tmRNA-mediated protein tagging and ribosome rescue. A ribosome stalls on an incomplete or untranslatable message, leading to (a) the recruitment of aminoacylated tmRNA to the ribosomal A site and (b) transfer of the nascent chain to the alanine-charged tRNA-like domain of tmRNA. A message-switching event (c) then replaces the faulty mRNA with an open reading frame within tmRNA (d), which is translated until a stop codon is reached (e) and the tagged protein is released and degraded by C-terminal specific proteases (f).

strated to be present in tmRNA (*6*). Both in vivo and in vitro experiments show this domain to be recognized and charged by alanyl-tRNA synthetase and required for association with 70S ribosomes (*6*−*9*). Moreover, mutations that affect recognition and charging by ala-tRNA synthetase impair ribosome association and peptide tagging activities (*6*, *8*−*11*). The aminoacylation determinants of tmRNA can be altered to those of histidyl-tRNA, and the altered tmRNA can be charged with histidyl-tRNA synthetase and incorporate histidine in in vitro poly-U-directed translation (*12*). The function of the mRNA-like domain of tmRNA came to light after the exceptional biochemical work and key observations of Tu et al. (*13*), who found that expression of IL-6 in *Escherichia coli* results in the production of both full-length protein and a series of incomplete protein fragments. Detailed analysis of these fragments revealed them to be derived from IL-6, but appended with an 11-amino acid AANDENYALAA C-terminal extension. A search for the mRNA encoding these amino acids revealed that 10 of the appended residues are encoded by sequences internal to tmRNA. Keiler et al. (*2*) combined the apparently disparate tRNA- and mRNA-like activities of this unusual RNA into a cohesive model (Figure 2). This model not only explained earlier observations but also provided a rationale for tmRNA activity and proposed consequences of this activity for the cell. Subsequent mutagenesis, biochemical, and structural studies are in full agreement with this model. The original *trans*-translation model (*1*, *2*) is described below.

trans-Translation Model for tmRNA Function. The *trans*-translation model for tmRNA function (Figure 2) postulates that alanine-charged tmRNA recognizes stalled ribosomes (a), binds like a tRNA to the ribosomal A-site, and donates its charged alanine to the nascent polypeptide chain in a standard transpeptidation reaction (b). tmRNA then acts as a surrogate mRNA, replacing the defective mRNA with the self-encoded peptide reading frame (c), to direct translation (*trans*-translation) of the degradation tag (d). Translation terminates normally at a stop codon provided by the mRNA-like domain of tmRNA (e). The final translation product of this process carries an 11-residue degradation tag at its C-terminus and thus becomes a substrate for C-terminal specific cellular proteases (e). All experimental evidence to date supports the basic tenets of the *trans*-translation model for tmRNA function.

Recent work from several laboratories has significantly expanded our understanding of the *trans*-translation process and has filled some of the gaps in our knowledge of this unique surveillance system. In the remainder of this review, we summarize our current understanding of tmRNA structure and function, its essential protein partners, and the role tmRNA and its cofactors play in recognition of stalled ribosomes, decay of defective mRNAs, and degradation of

tagged proteins. We also present an updated model that attempts to integrate the recently acquired knowledge of tmRNA and its protein cofactors in the *trans*-translation process.

Structure and Function of tmRNA Pseudoknots. Although the tRNA- and mRNA-like domains of tmRNA are the key functional elements, they constitute less than one-third of the RNA. The four pseudoknots, on the other hand, are the largest and most prominent features of tmRNA, yet their functional importance is not clearly understood. Initial studies indicated a functional role for PK1 in tmRNA function. Preliminary mutational studies of PK1 showed it to be important for both efficient aminoacylation and subsequent steps of the tagging process (*12*). NMR analysis of PK1 and the PK1 mutants confirmed the pseudoknot structure and showed structural changes in the mutants (*14*). Consistent with these conclusions, replacing PK1 with single-stranded RNA abolished both in vitro aminoacylation and tagging activities (*12*, *14*, *15*). These and related studies suggested a functional role for PK1 in tmRNA activity. However, a recent study suggests a sequence-independent structural role for PK1 (*16*). Detailed mutation and substitution analysis showed that replacing PK1 with sequences that form non-pseudoknot stem−loop structures yields functional tmRNA molecules in vivo, suggesting PK1 is involved in stabilizing tmRNA structure rather than participating directly in its function (*16*). Surprisingly, the presence of PK2, PK3, and PK4 in tmRNA was reported to be dispensable for *trans*-translation. Replacing any one of these three pseudoknots with a linear RNA sequence produced tmRNA variants that were efficiently aminoacylated and active in an in vitro tagging reaction (*15*). However, recent evidence contradicts these finding and suggests an important role for pseudoknots PK2−4 in folding and maturation of tmRNA (*17*). PK4, in particular, appears to be required for the in vivo activities of tmRNA. Clearly, much experimental work is required to reconcile these apparently contradictory findings and decipher the actual biological functions of these prominent and conserved features of tmRNA structure.

The structure of the ribosome shows a very tight fit of tRNAs, the P-site tRNA in particular, within the 70S ribosome, with contacts made by ribosomal RNA and protein from both the 30S and 50S subunits (*18*−*21*). It is not clear how tmRNA, which is roughly 4 times the size of a normal tRNA, fits into the same sites on the ribosome and participates in transpeptidation. It is also not clear how the four pseudoknots of tmRNA are accommodated during the various stages of *trans*-translation. It is possible that the pseudoknots remain outside of the ribosome during peptidyl transfer and translation of the tmRNA-encoded peptide reading frame. Alternatively, these structural elements might be unwound to facilitate decoding of the tmRNA peptide reading frame (*22*). Otherwise, accommodation of the additional bulk would require extraordinary conformational alteration of the ribosome. Additional biochemical and structural studies are required to provide further insights into how these pseudoknots contribute to the function of tmRNA at the various stages of the *trans*-translation process.

Essential Protein Factors. Three protein factors, alanyl-tRNA synthetase (Ala-RS), small protein B (SmpB), and elongation factor Tu (EF-Tu), are essential for the biological activity of tmRNA. Ala-RS has the simplest tRNA recognition requirements of all tRNA synthetases, requiring an amino acid acceptor stem (AA-stem) minihelix with a CCA 3′ end and a G·U wobble base pair at positions 3 and 70, respectively, of the stem structure (*23*). tmRNA fulfills this simple requirement, in that it has an AA-stem that possesses a G·U base pair at the corresponding positions (3 and 357, respectively) and ends with a CCA trinucleotide (Figure 1). Ala-RS is essential for tmRNA function as non-aminoacylated tmRNA is not recognized by EF-Tu and thus cannot be delivered to the ribosomal A-site. EF-Tu binds to the amino acid acceptor arm of the tRNA-like domain of tmRNA and protects the labile ester linkage of the aminoacylated molecule from hydrolysis (*24*−*26*). EF-Tu is also important for delivery and initial binding of tmRNA to stalled ribosomes and may also facilitate the structural rearrangement of the tmRNA molecule (*27*, *28*). Although some tRNA-like activity is observed in vitro for aminoacylated tmRNA in the absence of EF-Tu, the rate of the first transpeptidation reaction is far more efficient in the presence of EF-Tu (*29*, *30*).

The most significant protein partner of tmRNA is SmpB, a protein of roughly 160 residues that binds specifically and with high affinity to tmRNA (*31*). All known biological activities of tmRNA require SmpB (*1*, *3*, *17*, *24*, *30*−*48*). In *E. coli*, SmpB is not required for tmRNA expression or processing (*31*). Nevertheless, tmRNA does not associate stably with 70S ribosomes in cells lacking SmpB (*17*, *31*, *35*, *42*, *47*). Formation of the SmpB−tmRNA complex appears to be critical not only for ribosome recognition but also for one step, and perhaps several, after ribosome recognition (see below). A functional requirement for a fourth protein, ribosomal protein S1, remains a matter of debate (*49*, *50*). On the basis of some studies, S1 is thought to bind the mRNA-like domain, as well as the pseudoknot-rich domain, of tmRNA in a manner similar to that of other cellular mRNAs (*49*−*52*). Whether S1 function is required for tmRNA activity in vivo has not yet been adequately resolved. Although S1 function is essential in *E. coli*, several bacterial species lack the gene encoding canonical S1 protein.

Biochemical and Structural Insights into the SmpB−tmRNA Complex. Recently, a great deal of structural and biochemical information regarding SmpB protein and tmRNA has come to light. NMR solution structures of SmpB from *Aquifex aeolicus* (*48*) and *Thermus thermophilus* (*39*), and a cocrystal structure of *A. aeolicus* SmpB in complex with the tRNA-like domain of tmRNA (*53*), have been determined (Figure 3). NMR analysis of SmpB protein revealed the clustering of highly conserved amino acid residues on two discrete surfaces on opposite sides of the protein (*48*). One cluster coincides with the SmpB surface that recognizes nucleotides at the 3′ end of the D-loop in the tRNA-like domain of tmRNA [Figure 3A (*53*)]. Recent biochemical and structural studies have confirmed the importance of this cluster of conserved residues, identifying key amino acids that are essential for SmpB−tmRNA interactions [Figure 3B (*47*, *53*)]. Mutations in both *E. coli* and *Thermoanaetobacter tengcongensis* SmpB that affect these surface residues have adverse effects on the protein's ability to bind tmRNA with high affinity and specificity (*47*). The function of the second conserved cluster located on the opposite side of the tmRNA-binding surface has not been established (Figure 3C). Biochemical studies show that certain amino acid substitu-

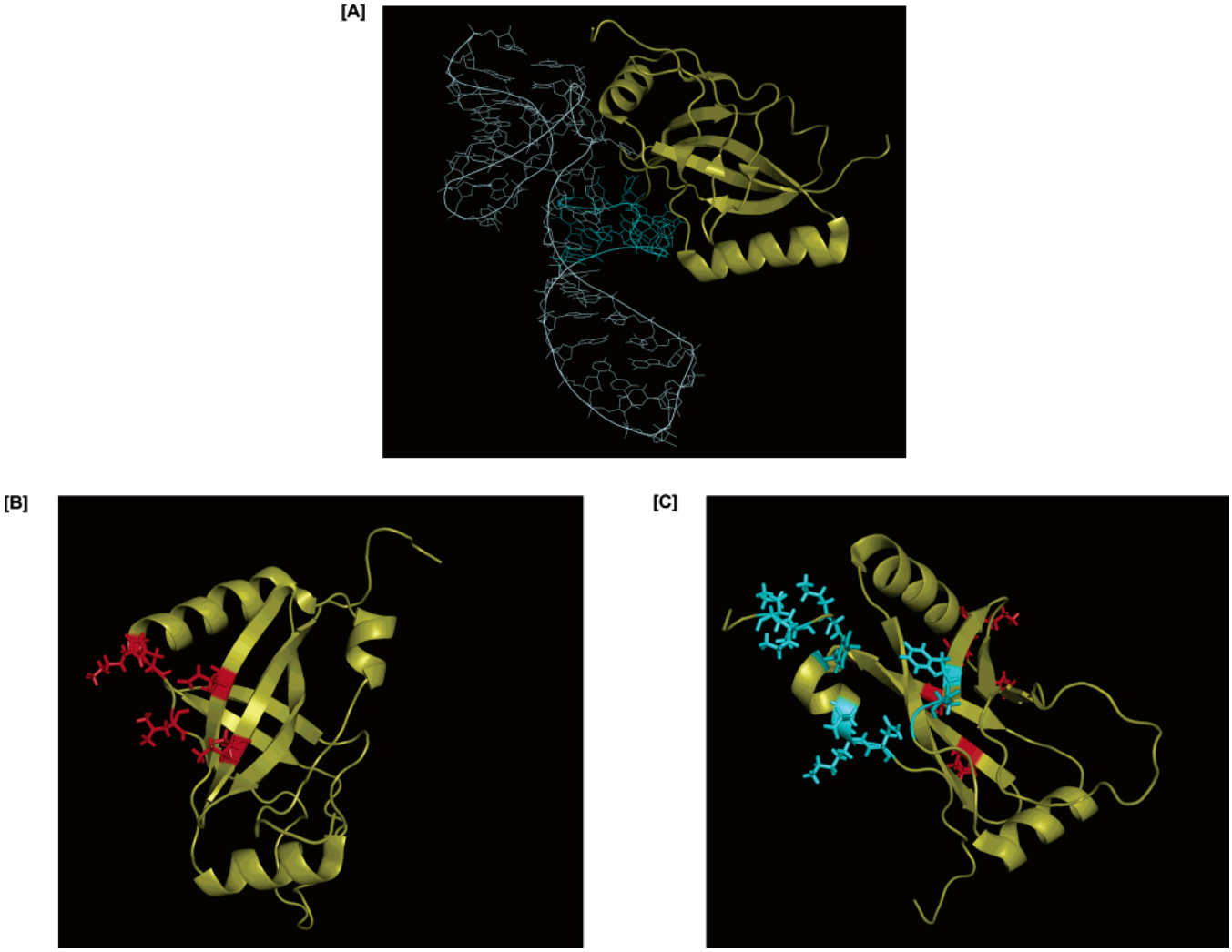

FIGURE 3: Ribbon diagram of SmpB structural models in complex with the tRNA-like domain of tmRNA (A) and alone (B and C). Panel A depicts SmpB bound to the D-loop (cyan) of the tRNA-like domain of tmRNA. SmpB-conserved amino acid residues are clustered into two regions on the surface of protein. Panel B depicts the positions of conserved amino acid residues (E26, L86, H89, and K119) on the surface of *A. aeolicus* SmpB that have been shown to play a crucial role in tmRNA binding. Panel C depicts positions of conserved amino acid residues (N12, K13, Y19, F51, K126, K128, K129, and R133) on the opposite side of the protein that are predicted to play a role in an SmpB function other than tmRNA binding, perhaps ribosome association. Figures were derived from Protein Data Bank entries 1P6V and 1K8H.

tions within this surface cluster have a profound effect on in vivo tagging activity, without affecting tmRNA binding (D. Dulebohn and A. W. Karzai, unpublished observations). It is at present unclear whether this cluster of conserved amino acids plays a role in ribosome recognition, accommodation of tmRNA in the A-site, or subsequent steps in the *trans*-translation process.

The core SmpB structure is quite similar in all three structural models. However, none of the models were able to discern the structure of the highly conserved C-terminal extension of SmpB protein. Sundermeier et al. (*35*) determined that the C-terminal tail of the SmpB protein performs a novel function that is critical in supporting the biological activity of tmRNA (Figure 4). Most interestingly, they found that SmpB variants in which this tail is truncated or mutated are still able to bind tmRNA and support stable association of the SmpB−tmRNA complex with stalled ribosomes (*35*). Nonetheless, these variants fail to support addition of the tmRNA-encoded tag, or even the transpeptidation step. These findings suggest that the C-terminal tail of SmpB is needed for a tmRNA activity downstream ribosome binding but prior to the first transpeptidation reaction that links the alanine charge to the nascent chain (*35*).

During normal translation, a ternary complex of charged cognate tRNA with EF-Tu and GTP is brought to the ribosomal A-site. Proper codon−anticodon interactions trigger conformational changes that activate the GTPase domain of EF-Tu. Rapid GTP hydrolysis is followed by release of EF-Tu and GDP and accommodation of the tRNA acceptor stem in the peptidyl transferase center. tmRNA lacks a traditional anticodon stem−loop structure, and thus, codon−anticodon interactions must a priori be absent from the tmRNA accommodation step. Like $tRNA^{Ala}$, tmRNA is recognized and charged by alanyl-tRNA synthetase (*6*), and the resulting ala-tmRNA forms a ternary complex with EF-Tu and GTP (*26*, *54*). Formation of a complex with EF-Tu and GTP protects the labile ester linkage of aminoacylated tmRNA (*26*), and by analogy with tRNAs, this interaction is also presumed to be important for delivery of tmRNA to stalled ribosomes. It is unclear what mechanistic events trigger GTP hydrolysis by EF-Tu when tmRNA is presented to the ribosomal A-site. It is clear that SmpB and EF-Tu are

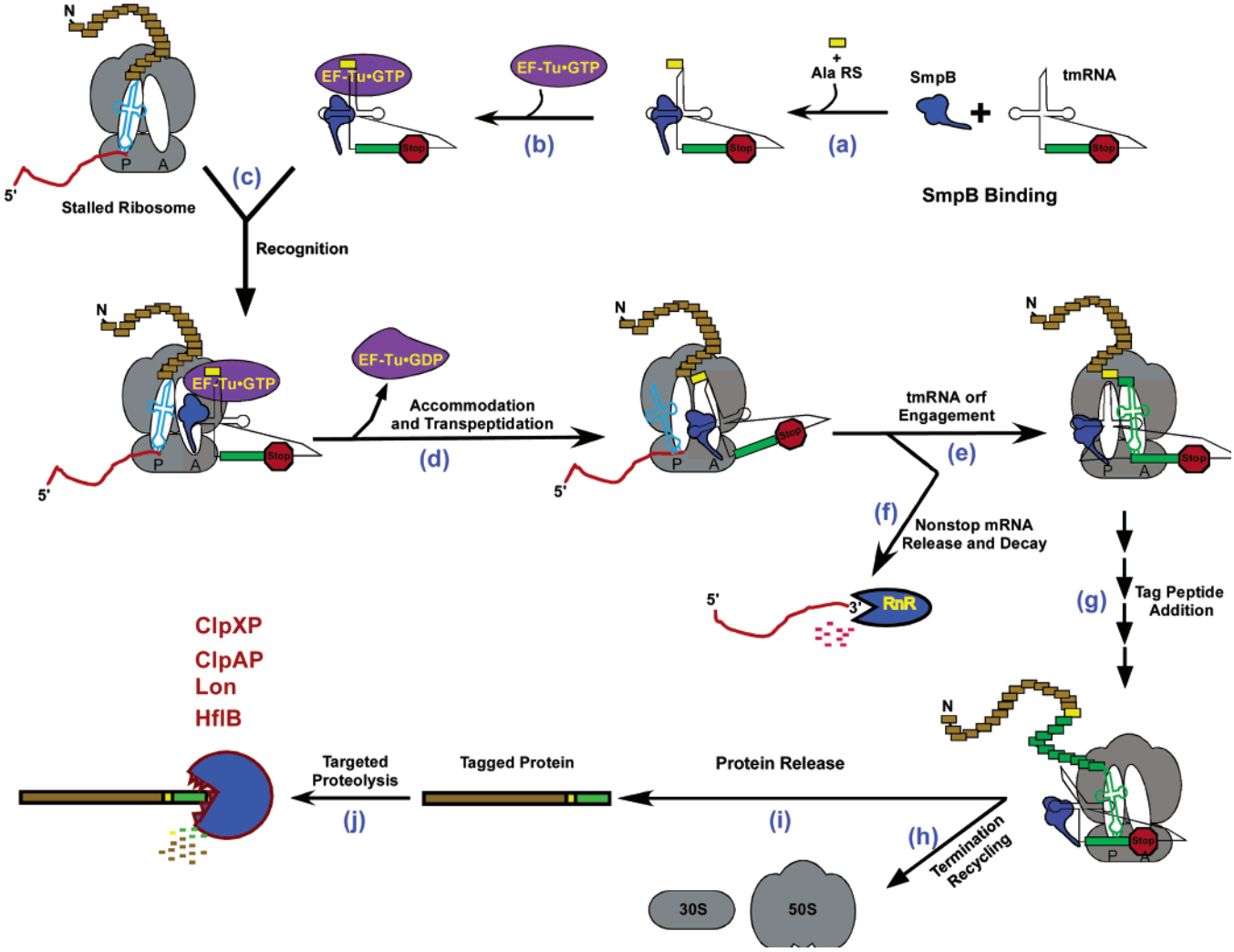

FIGURE 4: Integrated *trans*-translation model for SmpB−tmRNA function. Please see the text for details.

both required for productive association of tmRNA with stalled ribosomes. SmpB might serve to permit binding of tmRNA to stalled ribosomes, making the requisite contacts that elicit acceptance of tmRNA into the ribosomal A-site in the absence of codon−anticodon interactions. It is possible that the C-terminal tail of SmpB protein gains structure in the context of the ribosome to mediate specific contacts with ribosomal elements necessary for proper positioning and accommodation of tmRNA into the A-site. It is also possible that the C-terminal tail of SmpB plays a direct or indirect role in GTPase activation. Consistent with this inference, a recent study showed SmpB protein to be required for stimulating hydrolysis of GTP by EF-Tu in a ribosome- and tmRNA-dependent manner (*30*). It is suggested that SmpB might serve as a tRNA anticodon stem−loop mimic (*31*, *35*), and there is precedent for such a protein mimic in elongation factor G (*55*, *56*) and ribosome release and recycling factors (*57*). Alternatively, SmpB might mediate recognition of other ribosomal elements required for tmRNA-mediated function, facilitate some reactions directly, and/or serve a structural role in maintaining tmRNA in an active three-dimensional conformation. At present, there are no known SmpB functions that are independent of its role in supporting tmRNA activity.

Recognition of Stalled Ribosomes

The actual "signal" or conformational state that distinguishes an unproductively stalled ribosome from actively translating ribosomes is not known. Ribosomes stall if they reach the end of a transcript lacking a stop codon (*2*), encounter a stretch of rare codons (*58*, *59*), or encounter certain natural stall sequences (*60*−*65*). Regardless of the nature of the signal or conformational state of the ribosome (*1*), it is clear that recognition of stalled ribosomes requires alanyl-tmRNA in a quaternary complex with SmpB, EF-Tu, and GTP. SmpB protein function is essential for the recognition process. In the absence of SmpB, ala-tmRNA bound to EF-Tu and GTP is unable to stably associate with stalled ribosomes (*31*). A recent cryo-electron microscopy (cryo-EM) study evaluated the entry of tmRNA into a stalled ribosome (*27*). This study showed that in the pre-accommodated state the tRNA-like domain of tmRNA binds EF-Tu and engages the ribosomal A-site in a manner comparable to that of normal tRNAs (*27*). SmpB is modeled in the electron density map of the pre-accommodated state such that it simultaneously contacts the tRNA-like domain and the apical loop of helix H89 and is in the proximity of helices H69 and H71 in domain IV of 23S rRNA. The cryo-EM study also revealed that at this stage of *trans*-translation roughly two-thirds of tmRNA is not intimately

associated with the ribosome but appears to position the tmRNA peptide reading frame near the mRNA entrance to the A-site.

How is the tmRNA peptide reading frame established? Unlike canonical translation initiation, engagement of the tmRNA resume sequence appears not to require initiation factors or de novo dissociation and reassembly of the ribosomal subunits. The tmRNA amino acid acceptor stem presumably interacts with the peptidyltransferase center like a tRNA during transpeptidation, but the nature of subsequent events is unclear. Engagement of the tmRNA peptide reading frame into the mRNA channel of the rescued ribosome must a priori be different from engagement of all other mRNAs. For one, tmRNA function requires the ribosomal subunits to remain intact, in a 70S ribosome form with peptidyl-tRNA occupying the P-site and still base-paired with the last codon of the defective mRNA. Additionally, tmRNA does not possess a Shine-Delgarno ribosome binding site or a standard AUG start codon. Instead, a highly conserved GCA codon is utilized as part of the resume signal required to reinitiate translation with tmRNA as a template (Figure 1). The mechanistic details of how the tmRNA peptide reading frame is established have not yet been elucidated. What we do know is gleaned from mutagenesis studies of resume codon proximal sequences (*66*). These studies show that the first three nucleotides of the tmRNA peptide reading frame (the GCA resume codon) and a conserved trinucleotide (UAG) located two nucleotides upstream of the resume codon (Figure 1) are critical for establishing the reading frame. Mutations that alter these signals abolish both ribosome rescue and peptide tagging activities of tmRNA (*66*). The tmRNA peptide reading frame does contain a standard termination signal to permit normal recycling of ribosomes. The tmRNA ORF thus possesses the necessary information content for resumption of translation and termination or recycling of ribosomes.

Stoichiometry of the Ribosome Recognition Complex. The initial binding of tmRNA and its subsequent accommodation into the ribosomal A-site are activities intimately linked to SmpB function. Thus, which stages of *trans*-translation require SmpB function and how many SmpB molecules participate in this process are key questions that require further in-depth investigation. Currently available data suggest that a stalled ribosome recognition complex is composed of a single alanyl-tmRNA molecule, a single EF-Tu−GTP complex, and one or more SmpB molecules. The ambiguity in the number of functionally relevant SmpB molecules arises from in vitro studies suggesting that up to three SmpB molecules bind to a single tmRNA molecule and two SmpB molecules bind to a 70S ribosome, one to each 30S and 50S subunit (*29*, *40*, *46*). Although in vitro experiments suggest that several SmpB molecules can interact with a single tmRNA molecule, contacts made by the conserved RNA-binding surface of the protein with the high-affinity D-loop site of the tRNA-like domain (Figure 3) appear to be the most functionally important interactions (*3*, *24*, *27*, *31*, *35*, *47*, *48*, *53*). According to recent analysis of the interaction between tmRNA and SmpB, from *T. thermophilus* and *A. aeolicus* (*39*, *53*), only one SmpB binds to the D-loop equivalent of tmRNA (Figure 3A). A very surprising observation of another recent study, however, is that SmpB protein has higher apparent binding affinity for the 70S ribosome, and its subunits, than for tmRNA (*46*). Ribosome-associated SmpB−tmRNA complexes have been purified from wild-type cells, presumably engaged in various stages of the *trans*-translation process. In these samples, SmpB remains bound to 70S ribosome−tmRNA complexes. Following purification of these complexes and dissociation of the 70S ribosomes into 30S and 50S subunits, the bulk of SmpB protein remains associated with tmRNA, suggesting that the highest-affinity and -specificity partner of SmpB protein is tmRNA (*46*).

Initial cryo-EM-derived models of the complex formed by SmpB, tmRNA, EF-Tu, GDP, kirromycin, and 70S ribosomes (*27*) suggested the presence of a single SmpB protein in the pre-accommodated complex. A more recent cryo-EM study (*28*), however, suggests the presence of two SmpB molecules in each tmRNA-bound ribosome, with one SmpB oriented toward the A-site decoding center and the second molecule positioned in the 50S subunit. Both SmpB molecules interact with the tRNA-like domain of tmRNA, SmpB-1 interacting with the D-loop equivalent and SmpB-2 interacting with the T-loop equivalent of the tRNA-like domain. The results of available footprinting, mutational, and structural studies are consistent with a high-affinity interaction of SmpB with the D-loop of tmRNA (*24*, *40*, *53*). An unexpected finding from this recently determined structural model is that the tRNA-like domain of tmRNA is oriented toward the tmRNA ORF such that it is in the proximity of ribosomal protein S19 and helix 34 of the 30S subunit (*28*). It is difficult to visualize how tmRNA could function as a tRNA with its tRNA-like domain facing the ORF and an SmpB molecule occupying the 50S A-site. The second SmpB molecule will surely interfere with formation of the peptide bond. It is conceivable that this structure also represents an intermediate step in the *trans*-translation process. Proper accommodation, prior to the first tmRNA-mediated transpeptidation step, would thus necessitate the departure of the second SmpB molecule, currently modeled to occupy the 50S subunit, and require major rearrangements of the amino acid acceptor arm of the tRNA-like domain. Clarification of these issues will require a thorough analysis of (i) the total number of tmRNA and SmpB molecules per cell under various conditions, both on and off the ribosome, and (ii) the effect of various ribosome purification protocols on the partitioning of SmpB among tmRNA and ribosomes. Additionally, higher-resolution structural studies of the pre- and post-accommodated states of the SmpB−tmRNA−ribosome complex will help shed further light on the number of SmpB molecules bound to tmRNA and the rescued ribosome complex.

Decay of the Defective mRNAS

Aberrant mRNAs that Cause Ribosome Stalling. In *E. coli*, mRNA transcripts have an average half-life of 2−3 min, and endoribonucleases or 3′-to-5′ exoribonucleases probably generate mRNA fragments continuously (*67*). Additionally, premature transcriptional termination, untimely cleavage by endoribonucleases, trimming by 3′-to-5′ exoribonucleases, and stop codon mutations could all result in production of nonstop mRNAs lacking in-frame stop codons. Ribosomes stalled on nonstop mRNAs make an ideal substrate for tmRNA-mediated tagging and have been the most extensively studied substrates. Translation from nonstop mRNAs

results in tagging at protein positions corresponding to the terminal encoded residues (*35*, *66*). It is becoming increasingly apparent that there are additional mRNA determinants that lead to ribosome stalling in the context of full-length mRNAs. Ribosome stalling and subsequent tagging have been described at internal positions on mRNAs (*37*, *58*, *60*−*65*, *68*). tmRNA-mediated tagging has been observed on mRNAs containing a string of rare arginine codons, on weak termination codons, and at proline residues preceding stop codons (*58*, *60*−*63*, *68*−*71*). Therefore, if prolonged ribosome stalling is sufficient to recruit tmRNA (*64*, *72*, *73*), then tagging might also occur when ribosomes stall at abasic or other damaged sites, pause at stable mRNA secondary structures, or stop because charged tRNA is scarce. In some cases (i.e., at rare codons or at termination codons), cleavage of the mRNA within the ribosomal A-site has been observed (*62*, *69*, *74*−*76*). The endonuclease responsible for this cleavage has not been identified. The endonucleolytic activity is perhaps catalyzed by prokaryotic toxin−antitoxin pairs or through an unknown ribosome-mediated mechanism. It is now clear that rare codon-containing transcripts are cleaved in a translation-dependent, and SmpB- and tmRNA-independent, reaction (*59*, *62*, *64*, *65*). Ribosomes stalled on these messages become a substrate for SmpB and tmRNA, presumably after transcript cleavage has occurred and the 3′ fragment has been released (*62*, *69*, *74*−*76*). The cleavage process thus generates a ribosome complex stalled on the newly generated 3′ end of the mRNA with a peptidyl-tRNA in the P-sites and an empty A-site devoid of both tRNA and mRNA.

It has been demonstrated that the length of the mRNA (i.e., the number of bases 3′ of the ribosomal P-site codon) is an important determinant for tagging efficiency in vitro (*76*). The rate of *trans*-transfer is reduced dramatically when nine additional nucleotides are included at the 3′ end of the mRNA, and the rate approaches zero with 15 additional nucleotides (*76*). Hence, mRNA cleavage seems to be a prerequisite for SmpB−tmRNA complex-mediated tagging at internal positions. It is unclear, however, what molecular events underlie this requirement. An attractive hypothesis is that mRNA sequence downstream of the ribosomal A-site codon blocks potential contacts required for proper interactions of the SmpB−tmRNA−EF-Tu−GTP quaternary complex with the ribosome. It is uncertain whether such interactions would be required for stimulation of GTP hydrolysis or for downstream events such as accommodation of the tmRNA acceptor stem into the peptidyltransferase center or the switch to the tmRNA peptide reading frame. If all tmRNA-mediated tagging ultimately occurs on ribosomes stalled at the 3′ end of mRNAs, then the absence of mRNA in the A-site and the lack of 3′ untranslated sequence in the ribosomal mRNA channel could be key "signals" for tmRNA recognition of stalled ribosomes. Therefore, occupied A-site and the presence of 3′ untranslated sequence in the ribosomal mRNA channel might be determinants that prevent recognition of stalled ribosomes by tmRNA. This simple model is appealing as the A-site is always occupied during translation of "normal" stop codon-containing mRNAs. Furthermore, occupancy of the 3′ segment of the ribosomal mRNA channel by the untranslated 3′ end of mRNA could potentially explain why tmRNA does not interfere with routine translation.

Role of tmRNA in Enabling Nonstop mRNA Decay. As noted earlier, tmRNA has well-characterized tRNA- and mRNA-like functions that are required for ribosome rescue and protein tagging for directed proteolysis. Does tmRNA have any additional *trans*-translation-related functions? Several recent studies have demonstrated that tmRNA indeed performs a third key function, namely facilitating the degradation of the ribosome-stalling defective mRNA (*65*, *77*, *78*). One recent investigation (*77*), in addition to demonstrating a role for tmRNA in degradation of nonstop mRNAs, also revealed the identity of tmRNA sequence determinants responsible for this activity. These sequence determinants are located in the distal part of the tmRNA open reading frame, encoding the ultimate, penultimate, and antepenultimate amino acids of the peptide tag. Mutations that alter these tmRNA sequence elements have an adverse affect on the disposal of the nonstop mRNA, while leaving the tRNA and mRNA functions entirely unaffected. More significantly, specific mutations that change the nucleotide sequence of the peptide reading frame without altering the nature or identity of the peptide tag still exhibit the characteristic defect in nonstop mRNA decay. In contrast, mutations in codons 3−6 of the tmRNA open reading frame do not have an adverse affect on degradation of defective mRNAs. Therefore, in addition to its tRNA- and mRNA-like functions, tmRNA also plays an important role in promoting the decay of nonstop mRNAs.

How does tmRNA facilitate nonstop mRNA decay? The tmRNA-mediated nonstop mRNA decay is SmpB-dependent (*37*). This strict SmpB requirement suggests that the SmpB−tmRNA complex must be actively engaged with stalled ribosomes and in the early stages of the *trans*-translation process. The dependence of the nonstop mRNA decay on SmpB, tmRNA, and active translation of the target mRNA also suggests that RNase loading must occur prior to disengagement of the aberrant mRNA from the rescued ribosome or full engagement of the ribosome with the tmRNA reading frame. The following possible mechanisms for tmRNA-mediated degradation of nonstop mRNAs have been proposed (*37*, *77*). First, sequence elements within tmRNA, more specifically within the resume codon distal part of the tmRNA ORF, could be involved in interactions with ribosomal elements that promote binding, rearrangement, or loading of an RNase(s) onto the released defective mRNA. Second, this segment of tmRNA might be involved in interactions with other sequence elements in tmRNA or tmRNA-associated factors that then facilitate binding or loading of RNase onto the defective mRNA. A third possibility could be that this segment of tmRNA might interact directly with an RNase and deliver it to the defective message. Clearly, further work is required to distinguish among these possibilities.

RNase(s) Involved in Degradation of the Nonstop mRNAs. In *E. coli*, the three primary 3′-to-5′ RNases, PNPase, RNase II, and RNase R, are likely candidates for degradation of nonstop mRNAs. RNase R, in particular, is a likely candidate for targeted mRNA decay, as it was previously shown to be associated with a multicomponent protein−RNA complex that included SmpB protein and SsrA RNA (*51*). Additionally, in *Caulobacter crescentus*, RNase R was shown to be involved in the regulated degradation of the two-piece tmRNA in a cell cycle-dependent manner (*44*). Indeed, recent

evidence demonstrates that RNase R activity is essential for degradation of aberrant mRNAs in *E. coli* (*37*). This targeted RNase R activity is dependent on the presence of SmpB protein and tmRNA, suggesting that active *trans*-translation is required to engage RNase R with its intended targets. Although the exact timing of the release of defective mRNA from the rescued 70S ribosome has not been determined, it has been postulated that it is released from the rescued ribosome prior to engagement of the tmRNA peptide reading frame and resumption of translation with tmRNA as the surrogate template (*66*, *79*). Thus, RNase R must be engaged with the defective transcript at an early stage of the *trans*-translation process, most likely prior to the discharge of the faulty mRNA from the rescued ribosome. On the basis of these findings, RNase R appears to be an important component of the tmRNA-mediated translation surveillance system.

Degradation of tmRNA-Tagged Proteins

To date, the energy-dependent proteases ClpXP, ClpAP, and FtsH and the periplasmic energy-independent protease Tsp have been shown to degrade tmRNA-tagged peptides due to their recognition of the tmRNA tag sequence (*2*, *80*, *81*). However, these proteases do not degrade tmRNA-tagged peptides with equal prowess. ClpXP is considered to be responsible for the degradation of the bulk of tmRNA-tagged peptides in vivo. ClpAP has been shown to degrade tmRNA-tagged peptide constructs in vitro, but its contribution to in vivo disposal of tagged proteins is not as profound (*80*, *82*). The inner membrane-bound FtsH protease is restricted to protein substrates that are locally available and have low thermodynamic stability (*81*, *83*). Accordingly, FtsH has a narrower specificity than ClpXP and ClpAP. The degradation of tmRNA-tagged peptides by Tsp is dependent on their export to the periplasm (*2*). The ATP-dependent protease Lon was recently identified in a genetic screen, designed to find genes with *trans*-translation-related functions, and shown to be an important contributor to degradation of tmRNA-tagged proteins (J. Choy and A. W. Karzai, unpublished observations). Protein tagging and stability assays demonstrated that Lon participates in the degradation of tmRNA-tagged proteins. Therefore, the involvement of several major cellular proteases in the turnover of tmRNA-tagged proteins emphasizes the physiological significance of the degradative function of *trans*-translation.

The tmRNA peptide tag does not affect the structure or thermodynamic stability of the attached proteins (*80*), suggesting that it causes degradation simply by providing a recognition site for protease binding. The well-studied ClpXP and ClpAP proteases are multicomponent enzymes (*84*, *85*), in which ClpP, a tetradecameric serine protease, forms stacked heptameric rings that enclose a degradation chamber. The ClpX and ClpA subunits of the chaperone−peptidase complex are hexameric proteins, belonging to the Clp/Hsp100 chaperone family of ATPases that interact independently with ClpP to mediate substrate binding and regulate protease activity (*84*−*86*). A recent series of elegant experiments show that ClpX and ClpA recognize specific and distinct amino acid sequence elements of the tag peptide, unfold the tagged protein in an ATP-dependent manner, and feed it to the ClpP peptidase (*87*−*89*).

Are tmRNA-tagged polypeptides released and degraded normally, or is there a special mechanism that ensures rapid degradation? In recent years, a handful of proteins, termed specificity modulators or "specificity-enhancing factors", that modulate the activity of bacterial energy-dependent proteases have been described (*90*). The ability of these adaptor proteins to influence proteolytic capacity is impressive, with observations indicating stimulatory, inhibitory, and even redirecting effects (*86*). Of particular relevance to *trans*-translation is the discovery that SspB specifically binds to the tmRNA peptide tag and delivers tagged peptides to ClpXP for degradation (*87*). SspB has been shown to improve the targeting of tmRNA-tagged peptides to ClpXP, presumably favoring the degradation of tmRNA-tagged peptides that it preferentially binds. SspB binds to a region in the tmRNA tag that overlaps with the determinants recognized by ClpA (*88*). As a result, the association of SspB with a tmRNA-tagged peptide also exerts an inhibitory effect on the degradation of this peptide by ClpAP. SspB is known to associate with ribosomes (*87*). As such, SspB might bind tmRNA-tagged proteins as they emerge from the rescued ribosome and target them directly for degradation. This mode of coordinated action could also explain why proteins that are cotranslationally appended with the tmRNA-encoded tag are degraded faster than the same protein carrying a hard-coded tmRNA peptide tag (*80*).

An Integrated Trans-Translation Model for the Function of the SmpB−tmRNA Complex

The picture that emerges from these and related studies is a more expanded view of the *trans*-translation process. This view is the basis for an integrated model (Figure 4) that incorporates the newly acquired insights and fills some of the mechanistic gaps present in the original *trans*-translation model (Figure 1). In this model, SmpB protein binds with high affinity and specificity to tmRNA and the complex is aminoacylated by Ala-RS (a). The aminoacylated tmRNA−SmpB complex is recognized by EF-Tu and GTP (b) and forms a stalled ribosome recognition complex. Ribosomes stalled at the 3′ end of nonstop mRNAs are initially recognized by this quaternary complex in a pre-accommodated state (c). Proper accommodation of the tRNA-like domain of tmRNA into the ribosomal A-site is then triggered by contacts, perhaps elicited by the C-terminal tail of SmpB, that promote GTP hydrolysis on EF-Tu. Accommodation is followed by the first transpeptidation reaction that links the alanine charge of tmRNA to the incomplete polypeptide (d). tmRNA then switches from a tRNA-like mode to an mRNA-like mode, engaging the ribosome on its own peptide reading frame (e), concomitant with the displacement of the defective mRNA (f). The damaged mRNA is selectively recognized and degraded by RNase R, in an SmpB- and tmRNA-dependent manner. The rescued ribosome resumes translation with the tmRNA ORF as its surrogate template (g). Translation terminates on the tmRNA-encoded stop codon, permitting recycling of stalled ribosomes into the cellular pool (h). The nascent polypeptide, now marked with an 11-amino acid degradation tag, is released from the translation machinery (i) and specifically recognized and degraded by C-terminal specific cellular proteases (j). Therefore, this system provides an effective quality-assurance mechanism for dealing with the three potential problems posed by defective mRNAs.

Physiological Significance of the SmpB−tmRNA System

The *smpB* and *ssrA* genes are present in all bacteria examined to date (*1*, *91*, *92*). This system is not required for growth of *E. coli*, *Salmonella typhimurium*, *Bacillus subtilis*, *Yersinia pestis*, or *Yersinia pseudotuberculosis* under ideal in vitro growth conditions (*93*). In contrast, the SmpB−tmRNA system is essential for growth of pathogenic bacteria such as *Neisseria gonorrheae*, *Mycobacterium genitalium*, and *Mycobacterium pneumoniae* (*93*, *94*). It has been postulated that this system is essential for most, if not all, pathogenic bacteria in vivo since processes such as clearing stalled ribosomes and protein quality control might be more critical under adverse conditions where errors leading to ribosome stalling are far more frequent. This possibility could, for example, explain why the SmpB−tmRNA system is dispensable for normal growth of *S. typhimurium* but is required for the survival of this bacterium within macrophages (*95*, *96*). The most extensive analysis of the role of this system in bacterial survival and pathogenesis was conducted in the *Yersinia* species of pathogenic bacteria. Pathogenic *Yersinia* employ a type III secretion system to inject a lethal cocktail of effector proteins (Yops) into the host cell cytoplasm (*97*−*99*). The study by Okan et al. (*38*) demonstrated that the SmpB−tmRNA system is crucial for *Y. pseudotuberculosis* to survive under adverse growth conditions, such as oxidative and nitrosative stress, carbon or nitrogen starvation, and sublethal concentrations of antibiotics. Most significantly, this study showed that *smpB-ssrA* mutations render the bacterium avirulent and unable to cause lethal disease in a mouse infection model. Consistent with these observations, the authors demonstrated that the mutant strain is unable to proliferate in macrophages and exhibits delayed Yop-mediated host cell cytotoxicity. They also determined that the *smpB-ssrA* mutant suffered severe deficiencies in the expression and secretion of Yops, and that this defect was at the level of transcription. These findings highlight the significance of the SmpB−tmRNA system in bacterial pathogenesis and survival under adverse environmental conditions.

How might the SmpB−tmRNA system function under adverse conditions? A number of recent reports have suggested that the SmpB−tmRNA system plays a regulatory role in modulating gene expression by maintaining the requisite intracellular concentrations of some regulatory factors. These factors might include transcriptional activators and repressors (*1*, *91*). Experiments aimed at identifying endogenous proteins modified with tmRNA-encoded tags have revealed the identity of a number of regulatory proteins, including the LacI repressor (*68*, *71*), the λ-cI repressor, YbeL, GalE, and RbsK (*68*). In the case of the LacI repressor, it is proposed that an increase in its intracellular concentration results in the binding of LacI protein to secondary *lac* operator sequences located within the *lacI* gene. This binding is thought to lead to premature transcription termination and generation of truncated nonstop *lacI* mRNAs that, in turn, cause ribosome stalling and recognition by the SmpB−tmRNA system (*68*, *71*). The tmRNA-tagged LacI protein is proposed to undergo rapid degradation by cellular proteases. This type of negative feedback regulation is thought to maintain the desired concentration of LacI protein needed for sensitive tuning of physiological responses to the metabolic state of the cell. Indeed, *ssrA*$^-$ cells exhibit a marked delay in the induction of the *lac* operon, consistent with the notion that truncated and functional LacI repressor molecules accumulate in these cells, leading to repression of the *lac* operon.

A similar regulatory role is ascribed to the SmpB−tmRNA system in the bacteriophage Mu life cycle. Here the SmpB−tmRNA system is thought to play a role in maintaining the repressor population in a responsive state such that the phage can undergo de-repression (*31*, *100*). In an *ssrA* knockout strain, truncated but functional repressor molecules accumulate and render the phage unresponsive to conditions that trigger de-repression. Such a regulatory role of the SmpB−tmRNA system implies that tagging of some regulatory factors is a normal, constitutive process in the cell. Additionally, tagging of GalE and RbsK suggests the involvement of the SmpB−tmRNA system in regulation of carbon metabolism, which is consistent with the finding that an *smpB-ssrA* mutant exhibits slow recovery from carbon starvation (*38*). Further support for a potential regulatory role for the SmpB−tmRNA system comes from recent studies in *C. crescentus*, in which the optimal timing between the degradation of a specific response regulator, CtrA, and initiation of DNA replication is influenced by specific tagging and targeted degradation of the response regulator (*101*).

The SmpB−tmRNA system is thought also to affect gene expression solely through its ribosome rescue function (*93*). Although no direct evidence links the SmpB−tmRNA system to the expression and/or translation of a specific transcriptional activator, a number of reports point to the potential presence of such regulatory factors whose cellular concentrations are particularly sensitive to the balance between proteolysis (perhaps by Clp proteases in a tmRNA tagging-independent manner) and the translational efficiency of the cell (*93*, *100*, *102*). It is proposed that ribosome stalling, in the absence tmRNA, results in a substantial reduction in the degree of synthesis of these factors. Such a reduction in the level of protein synthesis coupled with proteolytic sensitivity is proposed to reduce the intracellular concentrations of these factors to a nonresponsive level in *smpB-ssrA* cells. This proposal is supported by the observation that deletion of Clp proteases partially compensates for the *λimmP22* phenotype of an *E. coli* ssrA mutant (*102*).

Summary

tmRNA is a unique molecule that orchestrates a fascinating biological quality-assurance system. This system has evolved to rescue unproductively stalled ribosomes and solve the potential problems associated with aberrant mRNAs. In association with the SmpB protein, tmRNA recognizes stalled ribosomes and tags the linked protein fragments for subsequent proteolytic degradation. C-Terminal specific proteases recognize, denature, and degrade proteins tagged by this system in both the cytoplasm and the periplasm. Additionally, this unique salvage system facilitates degradation of the defective mRNA by RNase R. Most importantly, the SmpB−tmRNA system appears to play key and physiologically important regulatory roles in bacterial virulence, fitness, and survival under adverse conditions. Although we have gained substantial insights into the general features and biological significance of this process, a great deal still needs to be

uncovered about the structural and mechanistic details of the molecular machines that mediate this unique pathway.

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REFERENCES

1. Karzai, A. W., Roche, E. D., and Sauer, R. T. (2000) The SsrA-SmpB system for protein tagging, directed degradation and ribosome rescue, *Nat. Struct. Biol. 7*, 449−455.
2. Keiler, K. C., Waller, P. R., and Sauer, R. T. (1996) Role of a peptide tagging system in degradation of proteins synthesized from damaged messenger RNA, *Science 271*, 990−993.
3. Withey, J. H., and Friedman, D. I. (2003) A salvage pathway for protein structures: tmRNA and trans-translation, *Annu. Rev. Microbiol. 57*, 101−123.
4. Williams, K. P., and Bartel, D. P. (1996) Phylogenetic analysis of tmRNA secondary structure, *RNA 2*, 1306−1310.
5. Felden, B., Himeno, H., Muto, A., Atkins, J. F., and Gesteland, R. F. (1996) Structural organization of *Escherichia coli* tmRNA, *Biochimie 78*, 979−983.
6. Komine, Y., Kitabatake, M., Yokogawa, T., Nishikawa, K., and Inokuchi, H. (1994) A tRNA-like structure is present in 10Sa RNA, a small stable RNA from *Escherichia coli*, *Proc. Natl. Acad. Sci. U.S.A. 91*, 9223−9227.
7. Ando, H., Kitabatake, M., and Inokuchi, H. (1996) 10Sa RNA complements the temperature-sensitive phenotype caused by a mutation in the phosphoribosyl pyrophosphate synthetase (prs) gene in *Escherichia coli*, *Genes Genet. Syst. 71*, 47−50.
8. Komine, Y., Kitabatake, M., and Inokuchi, H. (1996) 10Sa RNA is associated with 70S ribosome particles in *Escherichia coli*, *J. Biochem. 119*, 463−467.
9. Himeno, H., Sato, M., Tadaki, T., Fukushima, M., Ushida, C., and Muto, A. (1997) In vitro trans translation mediated by alanine-charged 10Sa RNA, *J. Mol. Biol. 268*, 803−808.
10. Muto, A., Sato, M., Tadaki, T., Fukushima, M., Ushida, C., and Himeno, H. (1996) Structure and function of 10Sa RNA: Trans-translation system, *Biochimie 78*, 985−991.
11. Himeno, H., Nameki, N., Tadaki, T., Sato, M., Hanawa, K., Fukushima, M., Ishii, M., Ushida, C., and Muto, A. (1997) *Escherichia coli* tmRNA (10Sa RNA) in trans-translation, *Nucleic Acids Symp. Ser.*, 185−186.
12. Nameki, N., Tadaki, T., Muto, A., and Himeno, H. (1999) Amino acid acceptor identity switch of *Escherichia coli* tmRNA from alanine to histidine in vitro, *J. Mol. Biol. 289*, 1−7.
13. Tu, G. F., Reid, G. E., Zhang, J. G., Moritz, R. L., and Simpson, R. J. (1995) C-Terminal extension of truncated recombinant proteins in *Escherichia coli* with a 10Sa RNA decapeptide, *J. Biol. Chem. 270*, 9322−9326.
14. Nameki, N., Chattopadhyay, P., Himeno, H., Muto, A., and Kawai, G. (1999) An NMR and mutational analysis of an RNA pseudoknot of *Escherichia coli* tmRNA involved in trans-translation, *Nucleic Acids Res. 27*, 3667−3675.
15. Nameki, N., Tadaki, T., Himeno, H., and Muto, A. (2000) Three of four pseudoknots in tmRNA are interchangeable and are substitutable with single-stranded RNAs, *FEBS Lett. 470*, 345−349.
16. Tanner, D. R., Dewey, J. D., Miller, M. R., and Buskirk, A. R. (2006) Genetic Analysis of the Structure and Function of Transfer Messenger RNA Pseudoknot 1, *J. Biol. Chem. 281*, 10561−10566.
17. Wower, I. K., Zwieb, C., and Wower, J. (2004) Contributions of Pseudoknots and Protein SmpB to the Structure and Function of tmRNA in trans-Translation, *J. Biol. Chem. 279*, 54202−54209.
18. Cate, J. H., Yusupov, M. M., Yusupova, G. Z., Earnest, T. N., and Noller, H. F. (1999) X-ray crystal structures of 70S ribosome functional complexes, *Science 285*, 2095−2104.
19. Yusupov, M. M., Yusupova, G. Z., Baucom, A., Lieberman, K., Earnest, T. N., Cate, J. H., and Noller, H. F. (2001) Crystal structure of the ribosome at 5.5 Å resolution, *Science 292*, 883−896.
20. Selmer, M., Dunham, C. M., Murphy, F. V. t., Weixlbaumer, A., Petry, S., Kelley, A. C., Weir, J. R., and Ramakrishnan, V. (2006) Structure of the 70S ribosome complexed with mRNA and tRNA, *Science 313*, 1935−1942.
21. Schuwirth, B. S., Borovinskaya, M. A., Hau, C. W., Zhang, W., Vila-Sanjurjo, A., Holton, J. M., and Cate, J. H. (2005) Structures of the bacterial ribosome at 3.5 Å resolution, *Science 310*, 827−834.
22. Wower, I. K., Zwieb, C., and Wower, J. (2005) Transfer-messenger RNA unfolds as it transits the ribosome, *RNA 11*, 668−673.
23. Chihade, J. W., Hayashibara, K., Shiba, K., and Schimmel, P. (1998) Strong selective pressure to use G:U to mark an RNA acceptor stem for alanine, *Biochemistry 37*, 9193−9202.
24. Barends, S., Karzai, A. W., Sauer, R. T., Wower, J., and Kraal, B. (2001) Simultaneous and functional binding of SmpB and EF-Tu-TP to the alanyl acceptor arm of tmRNA, *J. Mol. Biol. 314*, 9−21.
25. Barends, S., Wower, J., and Kraal, B. (2000) Kinetic parameters for tmRNA binding to alanyl-tRNA synthetase and elongation factor Tu from *Escherichia coli*, *Biochemistry 39*, 2652−2658.
26. Rudinger-Thirion, J., Giegâe, R., and Felden, B. (1999) Amino-acylated tmRNA from *Escherichia coli* interacts with prokaryotic elongation factor Tu, *RNA 5*, 989−992.
27. Valle, M., Gillet, R., Kaur, S., Henne, A., Ramakrishnan, V., and Frank, J. (2003) Visualizing tmRNA entry into a stalled ribosome, *Science 300*, 127−130.
28. Kaur, S., Gillet, R., Li, W., Gursky, R., and Frank, J. (2006) Cryo-EM visualization of transfer messenger RNA with two SmpBs in a stalled ribosome, *Proc. Natl. Acad. Sci. U.S.A. 103*, 16484−16489.
29. Hallier, M., Ivanova, N., Rametti, A., Pavlov, M., Ehrenberg, M., and Felden, B. (2004) Pre-binding of small protein B to a stalled ribosome triggers trans-translation, *J. Biol. Chem. 279*, 25978−25985.
30. Shimizu, Y., and Ueda, T. (2006) SmpB Triggers GTP Hydrolysis of Elongation Factor Tu on Ribosomes by Compensating for the Lack of Codon-Anticodon Interaction during Trans-translation Initiation, *J. Biol. Chem. 281*, 15987−15996.
31. Karzai, A. W., Susskind, M. M., and Sauer, R. T. (1999) SmpB, a unique RNA-binding protein essential for the peptide-tagging activity of SsrA (tmRNA), *EMBO J. 18*, 3793−3799.
32. Wower, J., Zwieb, C. W., Hoffman, D. W., and Wower, I. K. (2002) SmpB: A Protein that Binds to Double-Stranded Segments in tmRNA and tRNA, *Biochemistry 41*, 8826−8836.
33. Wower, J., Wower, I. K., Kraal, B., and Zwieb, C. W. (2001) Quality control of the elongation step of protein synthesis by tmRNP, *J. Nutr. 131*, 2978S−2982S.
34. Wiegert, T., and Schumann, W. (2001) SsrA-mediated tagging in *Bacillus subtilis*, *J. Bacteriol. 183*, 3885−3889.
35. Sundermeier, T. R., Dulebohn, D. P., Cho, H. J., and Karzai, A. W. (2005) A previously uncharacterized role for small protein B (SmpB) in transfer messenger RNA-mediated trans-translation, *Proc. Natl. Acad. Sci. U.S.A. 102*, 2316−2321.
36. Shimizu, Y., and Ueda, T. (2002) The role of SmpB protein in trans-translation, *FEBS Lett. 514*, 74−77.
37. Richards, J., Mehta, P., and Karzai, A. W. (2006) RNase R degrades non-stop mRNAs selectively in an SmpB-tmRNA-dependent manner, *Mol. Microbiol. 62*, 1700−1712.
38. Okan, N. A., Bliska, J. B., and Karzai, A. W. (2006) A Role for the SmpB-SsrA system in *Yersinia pseudotuberculosis* pathogenesis, *PLoS Pathog. 2*, 50−62 (e6).
39. Nameki, N., Someya, T., Okano, S., Suemasa, R., Kimoto, M., Hanawa-Suetsugu, K., Terada, T., Shirouzu, M., Hirao, I., Takaku, H., Himeno, H., Muto, A., Kuramitsu, S., Yokoyama, S., and Kawai, G. (2005) Interaction Analysis between tmRNA and SmpB from *Thermus thermophilus*, *J. Biochem. 138*, 729−739.
40. Metzinger, L., Hallier, M., and Felden, B. (2005) Independent binding sites of small protein B onto transfer-messenger RNA during trans-translation, *Nucleic Acids Res. 33*, 2384−2394.
41. Keiler, K. C., and Shapiro, L. (2003) TmRNA is required for correct timing of DNA replication in *Caulobacter crescentus*, *J. Bacteriol. 185*, 573−580.
42. Jacob, Y., Sharkady, S. M., Bhardwaj, K., Sanda, A., and Williams, K. P. (2005) Function of the SmpB Tail in Transfer-messenger RNA Translation Revealed by a Nucleus-encoded Form, *J. Biol. Chem. 280*, 5503−5509.
43. Ivanova, N., Pavlov, M. Y., Bouakaz, E., Ehrenberg, M., and Schiavone, L. H. (2005) Mapping the interaction of SmpB with

ribosomes by footprinting of ribosomal RNA, *Nucleic Acids Res. 33*, 3529−3539.
44. Hong, S.-J., Tran, Q.-A., and Keiler, K. C. (2005) Cell cycle-regulated degradation of tmRNA is controlled by RNase R and SmpB, *Mol. Microbiol. 57*, 565−575.
45. Hanawa-Suetsugu, K., Takagi, M., Inokuchi, H., Himeno, H., and Muto, A. (2002) SmpB functions in various steps oftrans-translation, *Nucleic Acids Res. 30*, 1620−1629.
46. Hallier, M., Desreac, J., and Felden, B. (2006) Small protein B interacts with the large and the small subunits of a stalled ribosome during trans-translation, *Nucleic Acids Res. 34*, 1935−1943.
47. Dulebohn, D. P., Cho, H. J., and Karzai, A. W. (2006) Role of conserved surface amino acids in binding of SmpB protein to SsrA RNA, *J. Biol. Chem. 281*, 28536−28545.
48. Dong, G., Nowakowski, J., and Hoffman, D. W. (2002) Structure of small protein B: The protein component of the tmRNA-SmpB system for ribosome rescue, *EMBO J. 21*, 1845−1854.
49. McGinness, K. E., and Sauer, R. T. (2004) Ribosomal protein S1 binds mRNA and tmRNA similarly but plays distinct roles in translation of these molecules, *Proc. Natl. Acad. Sci. U.S.A. 101*, 13454−13459.
50. Wower, I. K., Zwieb, C. W., Guven, S. A., and Wower, J. (2000) Binding and cross-linking of tmRNA to ribosomal protein S1, on and off the *Escherichia coli* ribosome, *EMBO J. 19*, 6612−6621.
51. Karzai, A. W., and Sauer, R. T. (2001) Protein factors associated with the SsrA·SmpB tagging and ribosome rescue complex, *Proc. Natl. Acad. Sci. U.S.A. 98*, 3040−3044.
52. Okada, T., Wower, I. K., Wower, J., Zwieb, C. W., and Kimura, M. (2004) Contribution of the second OB fold of ribosomal protein S1 from *Escherichia coli* to the recognition of TmRNA, *Biosci., Biotechnol., Biochem. 68*, 2319−2325.
53. Gutmann, S., Haebel, P. W., Metzinger, L., Sutter, M., Felden, B., and Ban, N. (2003) Crystal structure of the transfer-RNA domain of transfer-messenger RNA in complex with SmpB, *Nature 424*, 699−703.
54. Barends, S., Bjork, K., Gultyaev, A. P., de Smit, M. H., Pleij, C. W., and Kraal, B. (2002) Functional evidence for D- and T-loop interactions in tmRNA, *FEBS Lett. 514*, 78−83.
55. AEvarsson, A., Brazhnikov, E., Garber, M., Zheltonosova, J., Chirgadze, Y., al-Karadaghi, S., Svensson, L. A., and Liljas, A. (1994) Three-dimensional structure of the ribosomal translocase: Elongation factor G from *Thermus thermophilus*, *EMBO J. 13*, 3669−3677.
56. Nyborg, J., Nissen, P., Kjeldgaard, M., Thirup, S., Polekhina, G., Clark, B. F., and Reshetnikova, L. (1997) Macromolecular mimicry in protein biosynthesis, *Folding Des. 2*, S7−S11.
57. Selmer, M., Al-Karadaghi, S., Hirokawa, G., Kaji, A., and Liljas, A. (1999) Crystal structure of *Thermotoga maritima* ribosome recycling factor: A tRNA mimic, *Science 286*, 2349−2352.
58. Roche, E. D., and Sauer, R. T. (1999) SsrA-mediated peptide tagging caused by rare codons and tRNA scarcity, *EMBO J. 18*, 4579−4589.
59. Li, X., Hirano, R., Tagami, H., and Aiba, H. (2006) Protein tagging at rare codons is caused by tmRNA action at the 3′ end of nonstop mRNA generated in response to ribosome stalling, *RNA 12*, 248−255.
60. Hayes, C. S., Bose, B., and Sauer, R. T. (2002) Proline residues at the C terminus of nascent chains induce SsrA tagging during translation termination, *J. Biol. Chem. 277*, 33825−33832.
61. Hayes, C. S., Bose, B., and Sauer, R. T. (2002) Stop codons preceded by rare arginine codons are efficient determinants of SsrA tagging in *Escherichia coli*, *Proc. Natl. Acad. Sci. U.S.A. 99*, 3440−3445.
62. Hayes, C. S., and Sauer, R. T. (2003) Cleavage of the A Site mRNA Codon during Ribosome Pausing Provides a Mechanism for Translational Quality Control, *Mol. Cell 12*, 903−911.
63. Sunohara, T., Abo, T., Inada, T., and Aiba, H. (2002) The C-terminal amino acid sequence of nascent peptide is a major determinant of SsrA tagging at all three stop codons, *RNA 8*, 1416−1427.
64. Sunohara, T., Jojima, K., Tagami, H., Inada, T., and Aiba, H. (2004) Ribosome stalling during translation elongation induces cleavage of mRNA being translated in *Escherichia coli*, *J. Biol. Chem. 279*, 15368−15375.
65. Sunohara, T., Jojima, K., Yamamoto, Y., Inada, T., and Aiba, H. (2004) Nascent-peptide-mediated ribosome stalling at a stop codon induces mRNA cleavage resulting in nonstop mRNA that is recognized by tmRNA, *RNA 10*, 378−386.
66. Williams, K. P., Martindale, K. A., and Bartel, D. P. (1999) Resuming translation on tmRNA: A unique mode of determining a reading frame, *EMBO J. 18*, 5423−5433.
67. Ehretsmann, C. P., Carpousis, A. J., and Krisch, H. M. (1992) mRNA degradation in prokaryotes, *FASEB J. 6*, 3186−3192.
68. Roche, E. D., and Sauer, R. T. (2001) Identification of endogenous SsrA-tagged proteins reveals tagging at positions corresponding to stop codons, *J. Biol. Chem. 276*, 28509−28515.
69. Pedersen, K., Zavialov, A. V., Pavlov, M. Y., Elf, J., Gerdes, K., and Ehrenberg, M. (2003) The bacterial toxin RelE displays codon-specific cleavage of mRNAs in the ribosomal A site, *Cell 112*, 131−140.
70. Fujihara, A., Tomatsu, H., Inagaki, S., Tadaki, T., Ushida, C., Himeno, H., and Muto, A. (2002) Detection of tmRNA-mediated trans-translation products in *Bacillus subtilis*, *Genes Cells 7*, 343−350.
71. Abo, T., Inada, T., Ogawa, K., and Aiba, H. (2000) SsrA-mediated tagging and proteolysis of LacI and its role in the regulation of lac operon, *EMBO J. 19*, 3762−3769.
72. Garza-Sanchez, F., Janssen, B. D., and Hayes, C. S. (2006) Prolyl-tRNA(Pro) in the A-site of SecM-arrested ribosomes inhibits the recruitment of transfer-messenger RNA, *J. Biol. Chem. 281*, 34258−34268.
73. Collier, J., Bohn, C., and Bouloc, P. (2004) SsrA tagging of *Escherichia coli* SecM at its translation arrest sequence, *J. Biol. Chem. 279*, 54193−54201.
74. Christensen, S. K., and Gerdes, K. (2003) RelE toxins from Bacteria and Archaea cleave mRNAs on translating ribosomes, which are rescued by tmRNA, *Mol. Microbiol. 48*, 1389−1400.
75. Christensen, S. K., Pedersen, K., Hansen, F. G., and Gerdes, K. (2003) Toxin-antitoxin Loci as Stress-response-elements: ChpAK/MazF and ChpBK Cleave Translated RNAs and are Counteracted by tmRNA, *J. Mol. Biol. 332*, 809−819.
76. Ivanova, N., Pavlov, M. Y., Felden, B., and Ehrenberg, M. (2004) Ribosome rescue by tmRNA requires truncated mRNAs, *J. Mol. Biol. 338*, 33−41.
77. Mehta, P., Richards, J., and Karzai, A. W. (2006) tmRNA determinants required for facilitating nonstop mRNA decay, *RNA 12*, 2187−2198.
78. Yamamoto, Y., Sunohara, T., Jojima, K., Inada, T., and Aiba, H. (2003) SsrA-mediated trans-translation plays a role in mRNA quality control by facilitating degradation of truncated mRNAs, *RNA 9*, 408−418.
79. Ivanova, N., Pavlov, M. Y., and Ehrenberg, M. (2005) tmRNA-induced release of messenger RNA from stalled ribosomes, *J. Mol. Biol. 350*, 897−905.
80. Gottesman, S., Roche, E., Zhou, Y., and Sauer, R. T. (1998) The ClpXP and ClpAP proteases degrade proteins with carboxy-terminal peptide tails added by the SsrA-tagging system, *Genes Dev. 12*, 1338−1347.
81. Herman, C., Thevenet, D., Bouloc, P., Walker, G. C., and D'Ari, R. (1998) Degradation of carboxy-terminal-tagged cytoplasmic proteins by the *Escherichia coli* protease HflB (FtsH), *Genes Dev. 12*, 1348−1355.
82. Farrell, C. M., Grossman, A. D., and Sauer, R. T. (2005) Cytoplasmic degradation of ssrA-tagged proteins, *Mol. Microbiol. 57*, 1750−1761.
83. Herman, C., Prakash, S., Lu, C. Z., Matouschek, A., and Gross, C. A. (2003) Lack of a robust unfoldase activity confers a unique level of substrate specificity to the universal AAA protease FtsH, *Mol. Cell 11*, 659−669.
84. Wickner, S., Maurizi, M. R., and Gottesman, S. (1999) Posttranslational quality control: Folding, refolding, and degrading proteins, *Science 286*, 1888−1893.
85. Porankiewicz, J., Wang, J., and Clarke, A. K. (1999) New insights into the ATP-dependent Clp protease: *Escherichia coli* and beyond, *Mol. Microbiol. 32*, 449−458.
86. Baker, T. A., and Sauer, R. T. (2006) ATP-dependent proteases of bacteria: Recognition logic and operating principles, *Trends Biochem. Sci. 31*, 647−653.
87. Levchenko, I., Seidel, M., Sauer, R. T., and Baker, T. A. (2000) A specificity-enhancing factor for the ClpXP degradation machine, *Science 289*, 2354−2356.
88. Flynn, J. M., Levchenko, I., Seidel, M., Wickner, S. H., Sauer, R. T., and Baker, T. A. (2001) Overlapping recognition determinants within the ssrA degradation tag allow modulation of proteolysis, *Proc. Natl. Acad. Sci. U.S.A. 98*, 10584−10589.

89. Levchenko, I., Grant, R. A., Wah, D. A., Sauer, R. T., and Baker, T. A. (2003) Structure of a delivery protein for an AAA+ protease in complex with a peptide degradation tag, *Mol. Cell 12*, 365−372.
90. Dougan, D. A., Reid, B. G., Horwich, A. L., and Bukau, B. (2002) ClpS, a substrate modulator of the ClpAP machine, *Mol. Cell 9*, 673−683.
91. Withey, J. H., and Friedman, D. I. (2002) The biological roles of trans-translation, *Curr. Opin. Microbiol. 5*, 154−159.
92. Gueneau de Novoa, P., and Williams, K. P. (2004) The tmRNA website: Reductive evolution of tmRNA in plastids and other endosymbionts, *Nucleic Acids Res. 32*, D104−D108.
93. Huang, C., Wolfgang, M. C., Withey, J., Koomey, M., and Friedman, D. I. (2000) Charged tmRNA but not tmRNA-mediated proteolysis is essential for *Neisseria gonorrhoeae* viability, *EMBO J. 19*, 1098−1107.
94. Hutchison, C. A., Peterson, S. N., Gill, S. R., Cline, R. T., White, O., Fraser, C. M., Smith, H. O., and Venter, J. C. (1999) Global transposon mutagenesis and a minimal *Mycoplasma* genome, *Science 286*, 2165−2169.
95. Baumler, A. J., Kusters, J. G., Stojiljkovic, I., and Heffron, F. (1994) *Salmonella typhimurium* loci involved in survival within macrophages, *Infect. Immun. 62*, 1623−1630.
96. Julio, S. M., Heithoff, D. M., and Mahan, M. J. (2000) ssrA (tmRNA) Plays a Role in *Salmonella enterica* Serovar *typhimurium* Pathogenesis, *J. Bacteriol. 182*, 1558−1563.
97. Cornelis, G. R. (1994) Yersinia pathogenicity factors, *Curr. Top. Microbiol. Immunol. 192*, 243−263.
98. Hueck, C. J. (1998) Type III protein secretion systems in bacterial pathogens of animals and plants, *Microbiol. Mol. Biol. Rev. 62*, 379−433.
99. Cornelis, G. R., and Van Gijsegem, F. (2000) Assembly and function of type III secretory systems, *Annu. Rev. Microbiol. 54*, 735−774.
100. Ranquet, C., Geiselmann, J., and Toussaint, A. (2001) The tRNA function of SsrA contributes to controlling repression of bacteriophage Mu prophage, *Proc. Natl. Acad. Sci. U.S.A. 98*, 10220−10225.
101. Keiler, K. C., and Shapiro, L. (2003) tmRNA in *Caulobacter crescentus* is cell cycle regulated by temporally controlled transcription and RNA degradation, *J. Bacteriol. 185*, 1825−1830.
102. Withey, J., and Friedman, D. (1999) Analysis of the role of trans-translation in the requirement of tmRNA for λimmP22 growth in *Escherichia coli*, *J. Bacteriol. 181*, 2148−2157.

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