The tmRNA-tagging mechanism and the control of gene expression: a review

Barends, S.; Barend, K.; Wezel, G.P. van

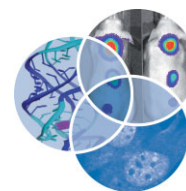**Note:** To cite this publication please use the final published version (if applicable).

The tmRNA-tagging mechanism and the control of gene expression: a review

Sharief Barends,[1] Barend Kraal[2] and Gilles P. van Wezel[2]*

The tmRNA-mediated *trans*-translation system is a unique quality control system in eubacteria that combines translational surveillance with the rescue of stalled ribosomes. During *trans*-translation, the chimeric tmRNA molecule—which acts as both tRNA and mRNA—is delivered to the ribosomal A site by a ribonucleoprotein complex of SmpB and EF-Tu–GTP, allowing the stalled ribosome to switch template and resume translation on a small coding sequence inside the tmRNA molecule. As a result, the aberrant protein becomes tagged by a sequence that is a target for proteolytic degradation. Thus, the system elegantly combines ribosome recycling with a clean-up function when triggered by truncated transcripts or rare codons. In addition, recent observations point to a specific regulation of the translation of a small number of genes by tmRNA-mediated inhibition or stimulation. In this review, we discuss the most prominent biochemical and structural aspects of *trans*-translation and then focus on the specific role of tmRNA in stress management and cell-cycle control of morphologically complex bacteria. © 2010 John Wiley & Sons, Ltd. *WIREs RNA* 2011 2 233–246 DOI: 10.1002/wrna.48

INTRODUCTION

Research activities on the structure and function of transfer-messenger RNA (tmRNA) started with its discovery in *Escherichia coli* in 1979 as 10Sa RNA, a small stable RNA of 363 nucleotides encoded by the *ssrA* gene.[1] It was postulated to have an open reading frame for a peptide of 25 residues.[2] A few years later it was found to have alanyl-tRNA-like properties in the stem-loop structures formed by its 5′ and 3′ ends.[3,4] Deletion of *ssrA* caused a phenotype of retarded cell growth and defective phage growth, although the molecular mechanisms remained unsolved for yet some time.[5] Studies with electrophoretic-mobility-shift assays showed interactions between tmRNA and transcriptional regulators such as the LacI, LexA, λcI, and P22-C1 proteins.[6]

A break-through was achieved through two crucial findings. First, the group of Simpson observed a nested set of truncated recombinant proteins in *E. coli*

that were fused to the C-terminal part of the putative 10Sa RNA-translation product, with an additional Ala residue inserted at the fusion site.[7] Second, the Sauer group recognized the 10Sa RNA-encoded tail peptide as a signal tag for degradation by cytoplasmic and periplasmic tail-specific proteases such as Tsp.[8] They drew the ground-breaking conclusion that 10Sa RNA functions as a tagging system for the degradation of proteins synthesized by ribosomes when stalled on mRNAs without stop codon (nonstop mRNA). By a mechanism called *trans*-translation, the stalled ribosome switches its decoding center from the truncated 3′ end of the mRNA to a specific internal site of alanyl-10Sa RNA at the ribosomal A site. After *trans*-peptidation to alanyl-10Sa RNA, translation is resumed and properly terminated on the reading frame. The rescue mechanism results in the release of stalled ribosomes and subsequent quality control by degradation of both truncated protein and damaged mRNA. The mechanism is illustrated in Figure 1. Because of its hybrid properties, 10Sa RNA became known as transfer-messenger RNA or tmRNA.[9]

tmRNA-mediated *trans*-translation has now become a standard subject in RNA biology and features in the modern text books. The impact of the subject is illustrated by the steady increase in

*Correspondence to: g.wezel@chem.leidenuniv.nl

[1]ProteoNic, Niels Bohrweg 11-13, 2333 CA Leiden, The Netherlands

[2]Molecular Biotechnology, Leiden Institute of Chemistry, Leiden University, PO Box 9502, 2300RA Leiden, The Netherlands
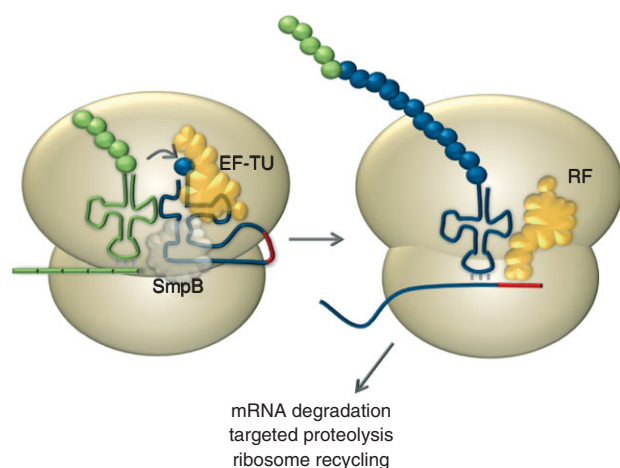
FIGURE 1 | The tmRNA-tagging mechanism. By virtue of the *trans*-translation mechanism's duality (ribosome release and protein tagging) nonfinished proteins become cotranslationally marked for destruction. The red line is the end of the reading frame of tmRNA, and represents the stop codon, to be recognized by the release factor (RF). For further explanation see text.

publications and citations from the discovery in 1979 via the second publication 10 years later, to reach a steady flow of around 50 publications per year in the last decade, which underlines the maturation of the field. Since this indicates the popularity of the subject, we have presented it graphically in Figure 2.
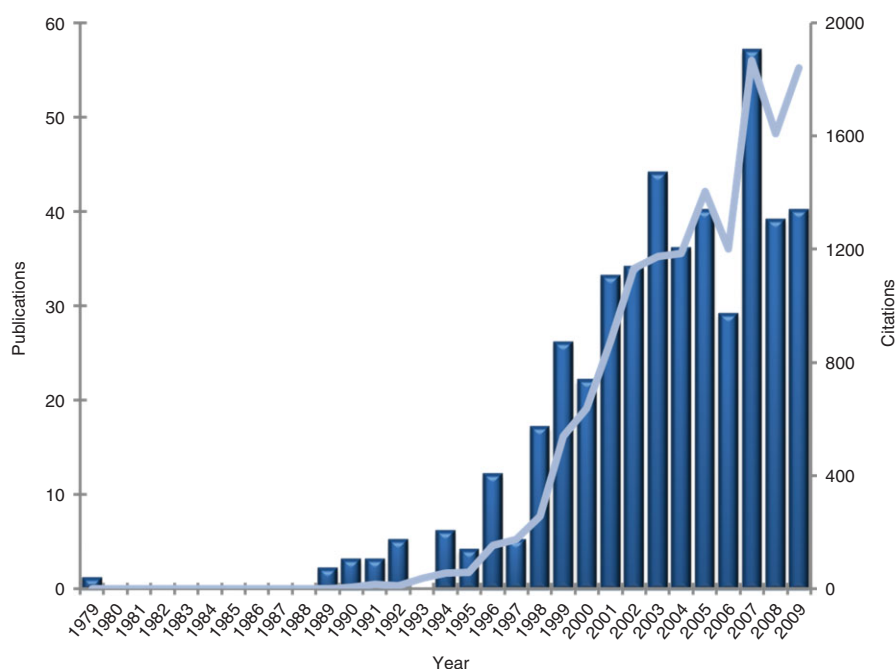
tmRNA AND *TRANS*-TRANSLATION

Universal Occurrence and Functionality of tmRNA in Eubacteria

The tRNA-like structure (TLS) of tmRNA (Figure 3) is conserved in all known eubacterial genomes, occasionally even as a two-piece complex, such as in α-proteobacteria like *Caulobacter crescentus*[10] and in some cyanobacteria, as a result of gene permutation and rearrangement.[11] The *ssrA* transcript is processed by tRNA maturation enzymes like RNase P, and is modified in the T loop for the display of 5-methyl-uridine and pseudo-uridine at the conserved tRNA positions.[12] Based on phylogenetic evidence and mutational analysis, interactions between its D- and T-loop could be demonstrated.[13] The latter interactions are conserved and important, not primarily for tmRNA alanylation and binding to EF-Tu, but rather for the proper functioning of the protein SmpB (small protein B).

The ribosome-rescue system usually operates well below capacity: the synthesis of about 0.4% of all proteins terminates with tagging during normal exponential growth of *E. coli*.[14] This tagging frequency increases substantially by nonstop mRNA overproduction, whereas the tmRNA concentration remains the same. Inactivation of *ssrA* diminishes the viability of cells under stress conditions. Interestingly,

FIGURE 2 | Trend of the number of publications (blue bars) and citations (light blue zig-zag line) of papers on tmRNA and *trans*-translation. Note that it took 10 years since the first publication in 1979[1] before the publications truly took off. Search terms were 'tmRNA', '*ssrA*' or '10Sa'. Data based on ISI Web of Science (year 2010 is not included).
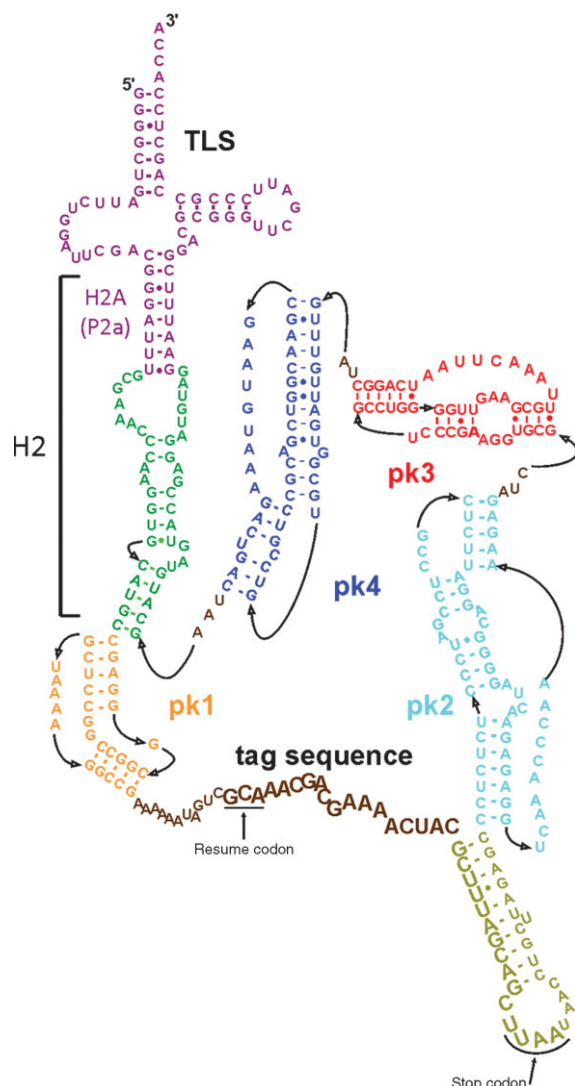
FIGURE 3 | Structure of tmRNA (10Sa RNA) from *E. coli* (363 nt). The TLS is given in purple, the pseudoknot structures PK1-4 in orange, cyan, red, and blue, respectively. Helix H2 (with section H2a, also called P2a) is the connection between the tRNA- and mRNA-like domains of tmRNA. The tag sequence with underlined resume and stop codon encodes the amino acid sequence ANDENYALAA.

Δ*ssrA* mutants are sensitive to subinhibitory concentrations of protein-synthesis inhibitors, whereas no increased sensitivity was measured to antibiotics unrelated to protein synthesis.[15] Further, in *Bacillus* tagging is strongly increased during growth at enhanced termperatures, strongly suggesting a relationship between heat shock and the activation of the *trans*-translation system.[16]

The Essential Protein SmpB and Ribosomal Protein S1 for tmRNA Scaffolding

In *E. coli*, the gene for tmRNA is preceded by *smpB*, encoding SmpB. The latter turned out to be essential for the tagging activity of tmRNA[17] and is also present in all known bacterial genomes. Formation of a tight complex between the two is indispensable for their stable association to a stalled ribosome and SmpB-defective bacteria have the same phenotype as tmRNA-defective ones. The conserved C-terminal tail of SmpB is essential for productive tmRNA accommodation in the ribosomal A site.[18] Crystallographic studies of SmpB in complex with the entire tRNA-like domain of tmRNA showed that SmpB mimics the anticodon arm (absent in tmRNA) of canonical tRNAs. The linker helix P2a (also called H2A) between tRNA- and mRNA-like domains of tmRNA mimics the long variable arm of bacterial class II tRNAs.[19] In addition, ribosomal protein S1 was reported to have a special affinity for tmRNA. It was found bound and cross-linked to the latter's mRNA domain and pseudoknot regions of pk2, pk3, and pk4.[19] *In vitro* studies with S1-free cell-free translation systems reported, that S1 is not essential for *trans*-translation.[20,21] *In vitro* and *in vivo* studies by others, however, led to a somewhat different conclusion. S1 is indeed dispensable for *trans*-peptidation of stalled peptidyl-tRNA to ribosome-bound Ala-tmRNA, but possibly required for switching the reading frame from the original mRNA to the mRNA domain of tmRNA.[22] Cryo-electron microscopy (cryo-EM) studies proposed a scaffolding role for SmpB and S1 in the binding of tmRNA at the 30S decoding center during the initial phase of *trans*-translation.[23,24]

A search for other protein factors associated with the tmRNA–SmpB complex yielded candidates like RNase R, PrsA, and SAF. Later, it was found that tmRNA levels are cell-cycle regulated by RNase R mediated degradation after proteolysis of SmpB. Intact SmpB functions as a protector of tmRNA.[25,26]

Kinetic Parameters for tmRNA Interactions

The interactions of tmRNA with alanyl-tRNA synthetase, EF-Tu, SmpB, and S1 were studied by analysis of the alanylation kinetics.[27] EF-Tu and SmpB bind simultaneously to the tRNA-like domain and enhance alanylation, whereas S1 binds independently to the mRNA-like domain and does not influence alanylation. On the ribosome, the rate of *trans*-peptidation of stalled peptidyl-tRNA to Ala-tmRNA depends on the mRNA length downstream of the P site. *In vitro* analysis showed a rapid decrease to about zero when this length exceeds five codons, whereas the *trans*-peptidation was strongly stimulated by RelE cleavage of the mRNA at its A-site codon.[28]

mRNA Cleavage in the A site of Pausing Ribosomes

As stated above, ribosomes poised at 3′ ends of damaged 'nonstop' mRNAs are substrates for tmRNA action. In addition, intact mRNAs that carry pausing ribosomes at internal sites may become subject to cleavage and eventual *trans*-translation.

The pausing on such intact 'no-go' mRNAs[29] may be caused by rare codons,[30] by inefficient translation termination (e.g., by a C-terminal proline or a LESG motif), or by sterical hindrance from adjacent or overlapping open reading frames.[31–33] Cleavage of 'no-go' mRNAs, in the ribosomal A site or a bit further downstream, turns them into nonstop mRNAs.[34–36] Indeed, *in vitro* experiments indicate that *trans*-translation requires the 3′ end of truncated mRNAs.[28] Apparently, the onset of *trans*-translation requires a codon-free ribosomal A site in order to accommodate the tmRNA–SmpB–EF-Tu complex. Cryo-EM analysis showed that the presence of a codon in the A site prevents the binding of SmpB.[23] Thus, the idea of a competition between Ala-tmRNA and regular aa-tRNA complexes or RFs for binding to an A-site codon can be excluded.

The endonuclease RelE, normally present as a toxin–antitoxin pair with RelB, was found to be responsible for codon-specific mRNA-cleavage in the A site.[11,37] RelE is activated during starvation conditions by Lon-mediated degradation of RelB. The 'toxic' effect of the cleavage is counteracted by *trans*-translation and ribosome rescue.[38] Actually, the term 'toxin' is misleading: RelE functions by lowering the global rate of translation in response to a shortage of aa-tRNAs. Recent crystallographic studies revealed why the ribosome is critical for RelE activity: the conserved nucleotide C1054 of 16S rRNA (we use *E. coli* numbering unless stated otherwise) is required for a correct orientation of the A-site codon in the active site of RelE.[39] RelE displays codon specificity, with UCG and CAG (for serine and glutamine, respectively), being the most rapidly cleaved sense codons.[37] Interestingly, there seems to be an evolutionary selection against tmRNA tag sequences carrying UCG and CAG codons (data not shown). This connects to a recent observation that in tmRNA there is a bias against ACA triplets, which is a target for the MazF toxin.[40]

Additionally, the activity of 3′ → 5′ exonucleases may play a role. For example, RNase II perhaps degrades mRNA to the downstream border of pausing ribosomes, thus facilitating RelE-independent cleavage of the A-site codon by a yet unknown RNase.[41] Interestingly, perturbations to A-site structure and decoding function, by mutations in ribosomal protein S12 or by the use of streptomycin, reduce A-site codon cleavage as well as tmRNA–SmpB activity.[42,43] The resulting nonstop mRNAs are substrates for eventual *trans*-translation and RNase R then degrades the defective nonstop mRNAs in an SmpB–tmRNA-dependent manner.[26]

Emerging Pictures of tmRNA on a Stalled Ribosome

Recent cryo-EM studies at 15–18 Å resolution provided further insight in the accommodation of tmRNA–SmpB in the ribosomal A site, after GTP hydrolysis on EF-Tu bound in the so-called pre-accommodation complex. A full-length Ala-tmRNA–SmpB complex was visualized at the A site after the release of EF-Tu–GDP.[44] It shows that SmpB mimics the anticodon- and D-stem of canonical tRNAs, stems that are missing in the tRNA-like domain of tmRNA. The transition from pre- to postaccommodation complex looks very similar to that of normal aa-tRNA, and results in a position of the mRNA domain closer to the decoding centre.

In Ref 45, the accommodation process was studied with a shortened Ala-tmRNA (tRNA-like domain with extended helix H2, no mRNA domain) in complex with two SmpB molecules and EF-Tu–GTP. Incubation with kirromycin kept the complex in the pre-accommodated state, without kirromycin it was found accommodated. While two SmpBs were bound per ribosome with kirromycin, only one remained in the accommodated state. The SmpB at the 30S decoding site remained in place after the dissociation of EF-Tu–GDP, whereas the one in interaction with the 50S subunit had disappeared. Relative to canonical translation, an additional movement was observed due to the rotation of H2. Perhaps such a movement would prepare the shift of the resume codon into the decoding center. Computer modeling studies[46] of the movement of tmRNA–SmpB in the ribosome during translocation of its tRNA-like domain from A to P site propose a rotational movement by which a structural element composed of SmpB with pseudoknot pk1 and loop A79-G87 of tmRNA moves from the ribosomal A site to the E site, around the tRNA-like domain in the P site. As a result, the resume codon (starting with G90) becomes precisely positioned at the A site.

RNA Context of the Resume Codon of the Tag Reading Frame

Since the discovery of the reading frame for *trans*-translation, the question has been asked which determinants would define the exact location of the resume codon. A Shine–Dalgarno motif as used for

translational initiation of most mRNAs is absent. Phylogenetic studies showed a strong preference for AUAG or AUAA at two nucleotides distance upstream of the resume codon GCN.[47] Mutations in this limited region could lead to −1 or +1 frame-shifts.[48–50] A mutant SmpB could restore a +1 shift.[51] Indeed, SmpB can interact with three conserved bases in the decoding center[52] and its flexible C-terminal tail is supposed to be close to U85 and the resume codon after accommodation in the A site.[53] Also ribosomal protein S1 could be close when bound to the A-rich region upstream of the resume codon, as appears from its UV-induced cross-link to U85.[22] S1 is known to form an essential part of the mRNA-binding track during translation initiation.[54] Further molecular details for the recognition of the resume frame are not yet available.

Degradation Pathways of tmRNA-tagged Proteins

After replacement of the nonstop mRNA by the messenger region of tmRNA in the ribosomal decoding center, a proper termination of translation can occur, followed by ribosome release. Protein products with C-terminal tmRNA-tags are degraded by tail-specific proteases such as Tsp, FtsH,[55] ClpXP and ClpAP,[56] and Lon.[57,58] The ribosome-associated SspB (discovered as a stringent starvation protein) markedly stimulates the ClpPX action[59,60] by recognition of tag residues 1–4 and 7.[61] It functions as a specificity enhancing adaptor between tag and ClpXP,[62,63] which is the major degradation protease for tmRNA-tagged proteins.[64] This may explain why mutant tags with a C-terminal Asp-Asp or (His)$_6$ substitution still induce some degradation of the tagged substrates.[14,59,65,66]

tmRNA and the Regulation of Transcription and Translation

Originally, tmRNA-mediated *trans*-translation has been discovered as a clean-up system for ribosomes jammed on damaged mRNAs. Soon thereafter the idea emerged that the *trans*-translation mechanism serves mainly as a release or a push for stuck ribosomes, from both truncated as well as intact mRNAs, rather than tagging truncated proteins.[67] A few examples where tmRNA exceeds its function as clean-up tool are discussed below.

The canonical *trans*-translation model dictates that ribosomes poised at 3′ ends of (truncated) mRNAs are substrates for tmRNA action. Otherwise, intact mRNAs that carry stalled ribosomes are cleaved prior to *trans*-translation. Thus, tmRNA action likely requires an empty ribosomal A site.[68] Indeed, *in vitro* experiments indicate that truncated mRNAs are better substrates for *trans*-translation,[28] whereas it is not yet possible to extrapolate these findings to the cellular situation. Interestingly, mRNA cleavage by so-called sequence-specific 'mRNA interferases'[37,69–71] and interferase-independent A-site cleavage[72] seem to operate in parallel to, and independent of, tmRNA tagging. Apparently, more than one mechanism is active for the release of stalled ribosomes, and it is a challenge to differentiate between them.

A well-studied example of tmRNA tagging serving the purpose of regulating gene expression is that concerning the lactose (*lac*) operon.[73] In the autoregulatory expression system for controlling levels of the transcriptional repressor LacI, high concentrations of LacI result in binding of LacI tetramers to two *lac* operators. One of the operators lies within the *lacI* coding region and binding of LacI to that site prevents transcription elongation, resulting in a nonstop *lacI* mRNA. In a tmRNA mutant, truncated forms of the LacI protein are produced and these incomplete LacI variants exhibit a dominant uninducible effect that results in a response delay once the inducer becomes available.[73] The LacI control mechanism where tmRNA deletion results in misregulation of a regulator might also apply to tmRNA-dependent development of bacteriophages.[74]

CELL-CYCLE CONTROL BY tmRNA

The role of tmRNA in the control of the *Caulobacter* cell-cycle

The first clear example of more specific involvement of tmRNA in cell-cycle control came from *C. crescentus*,[75,76] a rare example of a bacterium with a split gene for tmRNA, resulting in a two-piece complex. *Caulobacter* is a Gram-negative α-proteobacterium with two distinct cell types, a non-replicating 'swarmer' cell that uses a flagellum for movement and is suited for searching for nutrients, and a replicating 'stalked' cell that produces a long tubular stalk-like structure protruding from one pole. This dimorphic life cycle presents a selective advantage for growth in aqueous environments. The global response regulator CtrA coordinates DNA replication and cell division by repressing DNA replication initiation and transcription of the cell division gene *ftsZ* in swarmer cells.[77] In addition, CtrA mediates a DNA replication checkpoint of cell division by regulating the cell division genes *ftsQ* and *ftsA*.[78] The replication block during the G$_1$ cell-cycle phase coincides with the swarmer stage and when swarmer cells finally mature into a stalked cell, it is coordinated with a G$_1$-to-S

transition. CtrA is then degraded allowing for DNA replication to commence.[79] Concomitantly, tmRNA and SmpB levels increase, and decrease rapidly again early in the S phase.[25,75] This suggests that CtrA degradation and tmRNA tagging are coupled. However, in a tmRNA mutant there is still timely degradation of CtrA, but replication initiation is delayed. The start of replication involves most likely the proteolysis of other tagged proteins, since tmRNA variants where the tag has been mutated (resulting in a tag ending with a twin-aspartate ('tmRNA-DD') or a His-tag ('tmRNA-His') do not complement the phenotype, and even worsen the delay.[75,80] Recently, DnaA has been indicated as a possible mediator of the cell-cycle delay phenotype in a tmRNA mutant.[81]

From a wild-type strain expressing tmRNA-His, proteins tagged by tmRNA were purified and analyzed by proteomics. Of the 72 identified substrates only one target was tagged at a rare codon, and five targets seem to originate from problematic termination. Interestingly, some 70% of the tmRNA target genes in *C. crescentus* contain a nucleotide motif with the consensus CGACAAGATCGTCGTG located 3–60 codons upstream of the tagging site. The specificity of the element is underlined by the altered tagging pattern observed when the motif is moved to a different position in the gene. In further support, placing the motif in the green fluorescent protein (GFP)-encoding gene turned the otherwise intact GFP into a substrate for tmRNA tagging. The motif apparently dictates tagging at a remote site, and it is unclear whether it is preceded by mRNA cleavage.[80] It would be very interesting to see whether the tagged proteome and contributory nucleotide consensus motif differ between the stalked and swarmer stages of *C. crescentus*. Intriguingly, tmRNA localizes specifically at a specific stage of the *C. crescentus* life cycle.[82] As shown by *in situ* hybridization, tmRNA and SmpB colocalize to produce helical patterns in G1-phase cells, only to disappear at the onset of DNA replication. RNase R also forms spirals, but these are distinct from those of tmRNA-SmpB. It remains to be elucidated how wide-spread this specific localization is in bacteria, and what its contribution is to the function of *trans*-translation.

tmRNA and the control of sporulation

With the intriguing example of *Caulobacter*, it is clear that besides its canonical clean-up function tmRNA can play an important role in specific cell-cycle control. Another example of cell-cycle control was found recently in *Bacillus*, where tmRNA plays a role in the control of sporulation. Deletion of *ssrA* leads to a developmental arrest at the stage after the

formation of the forespore, which is primarily due to a block of *sigK* expression.[83] *sigK* is a gene that consists of two parts, *spoIIIC* and *spoIVCB*, separated by the so-called *skin* element. Removal of this element by the SpoIVCA recombinase results in joining of the two parts to generate full-length *sigK*.[84] It turns out that in the absence of tmRNA the level of SpoIVCA is reduced to about 10% of wild-type levels, leading to strongly reduced SigK activity, which results in a block in sporulation.[83] Thus *Bacillus* provides another exciting example of the role of tmRNA in the control of the bacterial cell cycle, although it must be noted that many proteins are tagged by tmRNA in this organism,[16] and therefore also here a major role for tmRNA lies in general surveillance.

A specific role for tmRNA in the control of *Streptomyces* development

The selectivity of tmRNA may be even more striking in streptomycetes, which are soil-dwelling antibiotic-producing filamentous bacteria with a life cycle not dissimilar from filamentous fungi.[85,86] Upon nutrient depletion the vegetative mycelium of streptomycetes initiates a complex development with an aerial mycelium that eventually produces chains of spores. The soil is a competitive habitat and organisms face stresses such as heat, desiccation, competing organisms and famine, and the latter is an important trigger for development and antibiotic production.[87] Possible involvement of tmRNA in stress management was highlighted by the direct dependence of *ssrA* transcription on the oxidative stress sigma factor σ^R,[88] and later tmRNA was shown to play a role in antibiotic resistance.[65,89–91] While initial mutational analysis of *Streptomyces lividans* revealed few changes to growth and development,[89] it soon turned out that the lack of tmRNA severely compromised stress resistance of streptomycetes, with enhanced sensitivity to, for example, heat shock and antibiotic resistance.[65,92] Growth is also compromised, and *ssrA* null mutants of *Streptomyces coelicolor*—a close relative of *S. lividans* and the best studied streptomycete—produce much smaller colonies[65] (Figure 4). While *trans*-translation is restored to *ssrA* mutants by the expression of wild-type tmRNA or tmRNA-His, the growth defects are not (see 'Conclusion' section at the end). Deletion of *ssrA* also has its repercussions for sporulation, which is almost completely abolished, indicating the importance of tmRNA for development[65,92] (Figure 5).

The control of *Streptomyces* development is complex, and while many regulatory networks have been uncovered at the transcriptional level,[93] little is known of mechanisms that effect control
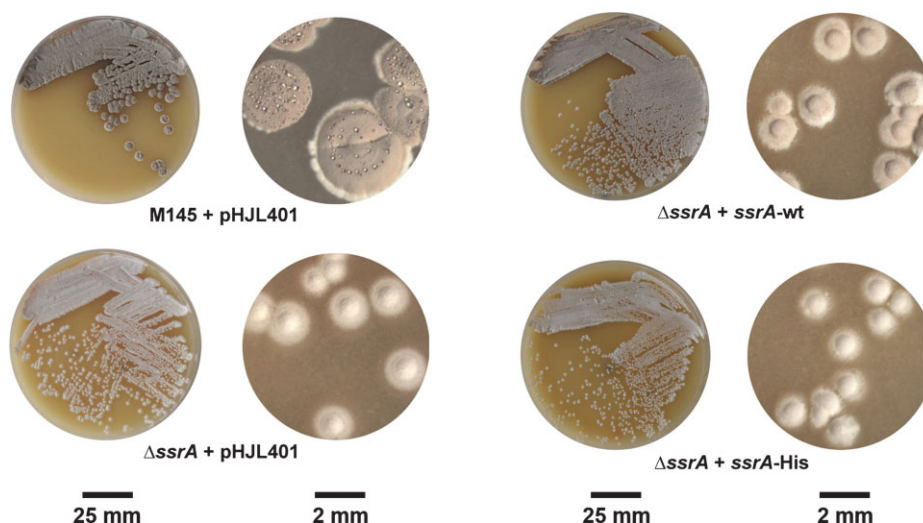
FIGURE 4 | Colony morphology of *ssrA* mutants (Δ*ssrA*) of *S. coelicolor* M145 and complementation by *ssrA in trans*. Deletion of *ssrA* leads to strongly reduced growth rate and colony size, and while *trans*-translation is restored by the introduction of both wild-type *ssrA* and recombinant *ssrA*-His, growth rate is not. However, sporulation (visible as gray pigmentation) is restored. Transformants: pHJL401, control plasmid without *ssrA*; ssrA-wt, pHJL401 harboring wild-type *ssrA*; ssrA-His, pHJL401 harboring recombinant *ssrA* specifying tmRNA-His. Images were taken by stereo microscopy, and shown at two magnifications as indicated by the mm bars.

development at the translational level. However, one very interesting example relevant for this review is that of a rare tRNA for the leucine codon UUA. The codon usage of the high G + C streptomycetes (around 72%) has evolved such that this codon only occurs around 150 times in the genome of the model streptomycete *S. coelicolor*[94] and in particular in genes involved in development and secondary metabolism (notably antibiotic production). As a result, tRNA^{UUA} is not essential, but inactivation results in developmental arrest and block of antibiotic production.[95,96] Considering the role of tmRNA in rescuing ribosomes stalled at rare codons,[30] one would expect proteins produced from genes containing UUA codons to be a prime target for *trans*-translation. However, the latter group was not found tagged
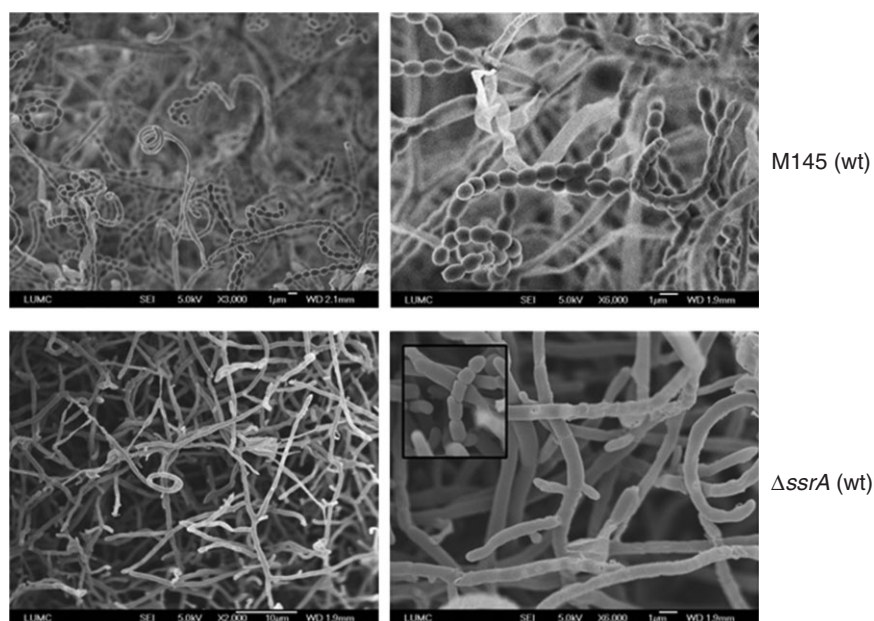

FIGURE 5 | Scanning electron micrographs demonstrating the sporulation defect of the *ssrA* mutant of *S. coelicolor*. Top row, wild-type strain M145; Bottom row, *ssrA* mutant. Note that many aerial hyphae of the *ssrA* mutant produce branches, which is never seen in wild-type hyphae. Inset: rare example of a spore chain.

by tmRNA, which rather surprisingly implies that either ribosomes do not pause long at UUA codons or pausing at UUA is not sufficient to trigger the tmRNA-mediated rescue system. This was the first indication that, despite its role in stress management, tmRNA may not function in a canonical fashion in streptomycetes.[65,89,92]

The big surprise came when the tmRNA-tagged proteome of *S. coelicolor* was analyzed using a recombinant tmRNA that results in His$_8$-tagged proteins; this revealed no more than 10 specific tmRNA targets (mostly tagged at or near the C-terminal end) on a predicted total proteome of around 7800 proteins.[65] Despite this specificity, proteins tagged by tmRNA in *Streptomyces* are also degraded rapidly. Almost all of the identified tmRNA targets are known cell-cycle or stress-related proteins: the pleiotropic nutrient sensor DasR,[97] the stress-inducible alternative elongation factor EF-Tu3,[98,99] the major chaperone DnaK (Hsp70),[100,101] the antibiotic-inducible protein TipA[102] and the cell-cycle regulators SsgA, SsgR, and SsgF.[103–105] In *S. lividans*, the tagging pattern was very similar to that in *S. coelicolor*, corroborating the target-specificity. Interestingly however, tagging was massively enhanced in *S. lividans* TK24, whose ribosomes display hyper-accuracy due to the presence of a streptomycin-resistant variant of ribosomal protein S12.[106] This strongly suggests that in streptomycetes slow ribosomes induce the tmRNA tagging system (S.B. and G.V.W., unpublished data). Intriguingly, and in contrast to this observation, *E. coli* strains carrying streptomycin-resistant S12 variants show reduced tagging capacity that could partially be alleviated by streptomycin,[43] highlighting another obvious difference between the two bacterial systems.

Western analysis showed that the expression of the critical stress and cell-cycle proteins DnaK and DasR depends on tmRNA, with the proteins almost completely absent in *ssrA* null mutants. Since no difference was observed between wild-type and mutant cells at the transcriptional level, this dependence apparently occurs exclusively at the translational level. Additionally, only a small percentage of the total cellular DasR or DnaK becomes tagged. This implies that the tagging of a few proteins is required for the accumulation of the total pool. This reveals an entirely new role for tmRNA, namely as a positive regulator of translation. Excitingly, translation of *eGFP* also becomes dependent on tmRNA when its stop codon is replaced by the 3′ UTR (including the last 60 codons) of *dasR*, strongly suggesting that the 'signal' for tmRNA tagging is embedded in that specific UTR.[65] How to explain these observations? It is known

that the tmRNA mechanism requires a truncated mRNA for the accommodation of tmRNA–SmpB in an empty ribosomal A site. Hence, if the 3′ UTR forces ribosome stalling, this must result in mRNA cleavage downstream of the *dasR* stop codon, as indicated by the observed tagging. The 3′ UTR might be somehow involved in an interaction that causes ribosome stalling on a downstream reading frame located close to *dasR*, with its RBS and start codon included in the 3′ UTR that is sufficient to trigger tagging when fused to the gene for GFP. By translational coupling, efficient termination at the *dasR* stop codon would become hampered, leading to DasR tagging. Ribosome rescue at the downstream reading frame and degradation of the impeding RNA interaction would result in efficient termination and a rapid burst of protein production from the upstream *dasR* cistron. The advantage of such a mechanism would be that preloaded *dasR* polysomes are created, allowing a rapid burst of protein expression from translation-committed mRNAs. The model awaits further experimental support and other explanations may also be envisaged.

Specific recruitment of tmRNA

Recently, yet another interesting trigger for tmRNA tagging has been postulated.[42] In this model, cotranslationally misfolded proteins enforce ribosome pausing and thus tmRNA tagging. Accordingly, tagging is significantly increased during heat shock and upon deletion of the ribosome-associated chaperone DnaK. Cotranslational misfolding and consequent tagging by tmRNA would specifically apply to multi-domain proteins. Suggestively, DnaK translation is controlled specifically by tmRNA in both *S. coelicolor* and *S. crescentus*.[65,80] During stress, such as heat shock, tmRNA is induced while high concentrations of DnaK are needed in order to deal with increased levels of misfolded proteins. While tmRNA aids in an instant burst of DnaK expression,[65] tmRNA and DnaK together tackle stalled ribosomes that emerge during stress; DnaK acts as a chaperone and diminishes misfolding of emerging nascent polypeptide chains while tmRNA frees stalled ribosomes and tags misfolded proteins that are beyond remedy (Figure 6).

CONCLUSION

The tmRNA-mediated *trans*-translation system plays a crucial role as a clean-up system in bacteria, which must have evolved as a means to recycle ribosomes stalled on the mRNA and at the same time remove the aberrantly synthesized (truncated) proteins, a mechanism that is particularly important
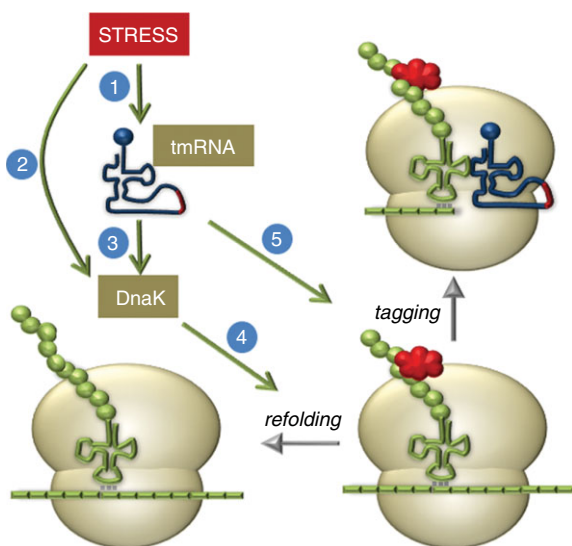
FIGURE 6 | tmRNA tagging controls misfolded protein levels. Upon stress, tmRNA (1) and, *via* RpoH, *dnaK* levels (2) are increased.[88,107,108] In turn, tmRNA stimulates DnaK translation (3)[65] and frees stalled ribosomes (5).[8] Misfolded proteins may force ribosome stalling,[42] and DnaK aids in the cotranslational (re-)folding of nascent peptide chains, allowing the process to continue normally (4).

under conditions of stress. Evidence is accumulating that tmRNA is also used to control the expression of specific genes, such as the control of the lactose operon in *E. coli*, and more generally in the control of development of *Caulobacter* and *Streptomyces*. Development may be seen as a continuous stress condition, and this is most obvious in streptomycetes. During sporulation of this complex microorganism, the vegetative mycelium is degraded in an autolytic process known as programmed cell death, releasing building blocks required for the build-up of the spore-forming aerial mycelium.[87,109] The strong increase in degraded RNA may already be sufficient to trigger the tmRNA system. However, the growth-phase dependent activity of tmRNA is probably a much more common phenomenon, illustrated by the involvement of tmRNA in ensuring sufficient expression of the stationary-phase sigma factor σ^S (RpoS) in *E. coli*.[110] The condition of famine—a major and common trigger of microbial development—leads to a decline in ribosomal fidelity at this stage of the life cycle, which contributes to the accumulation of the stress-response regulator σ^S.[111] So the involvement of tmRNA in developmental control in some more complex bacteria may not be so surprising as it may seem at first sight. Perhaps more intriguing is that in the specific examples of *Caulobacter* and *Streptomyces*, tmRNA displays rather strong target selectivity. The molecular basis for this is still largely unclear and needs to be investigated in more detail.

For a better understanding of the duality of the tmRNA tagging system, more targets need to be discovered. A possible new function for tmRNA might be related to bacterial fitness and defence against phage infection. In the newly discovered bacterial and archeal adaptive immunity system CRISPR (clustered regularly interspaced short palindromic repeats), small RNAs target invading viruses and foreign plasmid DNA in a fashion reminiscent of the eukaryotic RNAi system.[112–114] Both DNA and RNA seem to be target of the small guide RNAs resulting in cleavage of the target strand; a potential target for tmRNA tagging. It would be interesting to investigate whether CRISPR-mediated immunity and tmRNA tagging cooperate for ameliorating bacterial fitness during phage infection. Both CRISPR and tmRNA tagging are likely to have evolutionary evolved separately, since tmRNA is ubiquitously present in eubacteria, while CRISPR has been found in most archaea and 40% of the bacteria.

Finally, the function of the putative signaling peptide[115] encoded by a conserved overlapping reading frame—in opposite orientation—at the distal end of *ssrA* merits further examination. This observation has thus far received little or no attention, but may be rather important. For example, *trans*-translation is fully restored to *ssrA* deletion mutants by providing *ssrA* in *trans*, but only if the complementing sequence contains the wild-type gene including some flanking sequences. Wild-type tmRNA or tmRNA-His restore *trans*-translation, but defects such as reduced growth rate or higher susceptibility to antibiotics are not restored by tmRNA-His, and in some cases also not by wild-type tmRNA. Suggestively, in all cases where tmRNA failed to complement, expression of the putative divergently transcribed open reading frame (ORF) has been destroyed.

With this review we have attempted to provide a comprehensive overview of the biology of the intriguing small RNA molecule tmRNA. After its initial discovery and extensive structural and functional analysis, attention has recently been drawn toward more specific functions of tmRNA in the control of gene expression, and most notably cell-cycle control. Studies have been performed on tmRNA function in *Bacillus*, *Caulobacter*, and *Streptomyces*, but many other bacteria exist with complex life cycles, such as *Myxococcus* (forms intriguing swarming cells) or actinomycetes with a complex development but significantly different from *Streptomyces*, such as *Kineococcus* or *Frankia*. Study of such bacteria will undoubtedly give further valuable insight into the role of tmRNA in the specific control of gene expression.

REFERENCES

1. Ray BK, Apirion D. Characterization of 10S RNA: a new stable RNA molecule from Escherichia coli. *Mol Gen Genet* 1979, 174:25–32.

2. Chauhan AK, Apirion D. The gene for a small stable RNA (10Sa RNA) of Escherichia coli. *Mol Microbiol* 1989, 3:1481–1485.

3. Komine Y, Kitabatake M, Yokogawa T, Nishikawa K, Inokuchi H. A tRNA-like structure is present in 10Sa RNA, a small stable RNA from Escherichia coli. *Proc Natl Acad Sci USA* 1994, 91:9223–9227.

4. Ushida C, Himeno H, Watanabe T, Muto A. tRNA-like structures in 10Sa RNAs of Mycoplasma capricolum and Bacillus subtilis. *Nucleic Acids Res* 1994, 22:3392–3396.

5. Oh BK, Apirion D. 10Sa RNA, a small stable RNA of Escherichia coli, is functional. *Mol Gen Genet* 1991, 229:52–56.

6. Retallack DM, Friedman DI. A role for a small stable RNA in modulating the activity of DNA-binding proteins. *Cell* 1995, 83:227–235.

7. Tu GF, Reid GE, Zhang JG, Moritz JG, Simpson RJ. C-terminal extension of truncated recombinant proteins in Escherichia coli with a 10Sa RNA decapeptide. *J Biol Chem* 1995, 270:9322–9326.

8. Keiler KC, Waller PR, Sauer RT. Role of a peptide tagging system in degradation of proteins synthesized from damaged messenger RNA. *Science* 1996, 271:990–993.

9. Jentsch S. When proteins receive deadly messages at birth. *Science* 1996, 271:955–956.

10. Keiler KC, Shapiro L, Williams KP. tmRNAs that encode proteolysis-inducing tags are found in all known bacterial genomes: a two-piece tmRNA functions in Caulobacter. *Proc Natl Acad Sci USA* 2000, 97:7778–7783.

11. Williams KP. Descent of a split RNA. *Nucleic Acids Res* 2002, 30:2025–2030.

12. Felden B, Hanawa K, Atkins JF, Himeno H, Muto A, Gesteland RF, McCloskey JA, Crain PF. Presence and location of modified nucleotides in Escherichia coli tmRNA: structural mimicry with tRNA acceptor branches. *Embo J* 1998, 17:3188–3196.

13. Barends S, Bjork K, Gultyaev AP, de Smit MH, Pleij CW, Kraal B. Functional evidence for D- and T-loop interactions in tmRNA. *FEBS Lett* 2002, 514:78–83.

14. Moore SD, Sauer RT. Ribosome rescue: tmRNA tagging activity and capacity in Escherichia coli. *Mol Microbiol* 2005, 58:456–466.

15. de la Cruz J, Vioque A. Increased sensitivity to protein synthesis inhibitors in cells lacking tmRNA. *RNA* 2001, 7:1708–1716.

16. Fujihara A, Tomatsu H, Inagaki S, Tadaki T, Ushida C, Himeno H, Muto A. Detection of tmRNA-mediated trans-translation products in Bacillus subtilis. *Genes Cells* 2002, 7:343–350.

17. Karzai AW, Susskind MM, Sauer RT. SmpB, a unique RNA-binding protein essential for the peptide-tagging activity of SsrA (tmRNA). *Embo J* 1999, 18:3793–3799.

18. Sundermeier TR, Dulebohn DP, Cho HJ, Karzai AW. A previously uncharacterized role for small protein B (SmpB) in transfer messenger RNA-mediated trans-translation. *Proc Natl Acad Sci USA* 2005, 102:2316–2321.

19. Bessho Y, Shibata R, Sekine S, Murayama K, Higashijima K, Hori-Takemoto C, Shirouzu M, Kuramitsu S, Yokoyama S. Structural basis for functional mimicry of long-variable-arm tRNA by transfer-messenger RNA. *Proc Natl Acad Sci USA* 2007, 104:8293–8298.

20. Qi H, Shimizu Y, Ueda T. Ribosomal protein S1 is not essential for the trans-translation machinery. *J Mol Biol* 2007, 368:845–852.

21. Takada K, Takemoto C, Kawazoe M, Konno T, Hanawa-Suetsugu K, Lee S, Shirouzu M, Yokoyama S, Muto A, Himeno H. In vitro trans-translation of Thermus thermophilus: ribosomal protein S1 is not required for the early stage of trans-translation. *RNA* 2007, 13:503–510.

22. Wower IK, Zwieb CW, Guven SA, Wower J. Binding and cross-linking of tmRNA to ribosomal protein S1, on and off the Escherichia coli ribosome. *Embo J* 2000, 19:6612–6621.

23. Gillet R, Kaur S, Li W, Hallier M, Felden B, Frank J. Scaffolding as an organizing principle in trans-translation. The roles of small protein B and ribosomal protein S1. *J Biol Chem* 2007, 282:6356–6363.

24. Valle M, Gillet R, Kaur S, Henne A, Ramakrishnan V, Frank J. Visualizing tmRNA entry into a stalled ribosome. *Science* 2003, 300:127–130.

25. Hong SJ, Tran QA, Keiler KC. Cell cycle-regulated degradation of tmRNA is controlled by RNase R and SmpB. *Mol Microbiol* 2005, 57:565–575.

26. Richards J, Mehta P, Karzai AW, Nase R. Degrades non-stop mRNAs selectively in an SmpB-tmRNA-dependent manner. *Mol Microbiol* 2006, 62:1700–1712.

27. Barends S, Karzai AW, Sauer RT, Wower J, Kraal B. Simultaneous and functional binding of SmpB and EF-Tu-TP to the alanyl acceptor arm of tmRNA. *J Mol Biol* 2001, 314:9–21.

28. Ivanova N, Pavlov MY, Felden B, Ehrenberg M. Ribosome rescue by tmRNA requires truncated mRNAs. *J Mol Biol* 2004, 338:33–41.

29. Keiler KC. Biology of trans-translation. *Annu Rev Microbiol* 2008, 62:133–151.

30. Roche ED, Sauer RT. SsrA-mediated peptide tagging caused by rare codons and tRNA scarcity. *EMBO J* 1999, 18:4579–4589.

31. Collier J, Binet E, Bouloc P. Competition between SsrA tagging and translational termination at weak stop codons in Escherichia coli. *Mol Microbiol* 2002, 45:745–754.

32. Hayes CS, Bose B, Sauer RT. Stop codons preceded by rare arginine codons are efficient determinants of SsrA tagging in Escherichia coli. *Proc Natl Acad Sci USA* 2002, 99:3440–3445.

33. Roche ED, Sauer RT. Identification of endogenous SsrA-tagged proteins reveals tagging at positions corresponding to stop codons. *J Biol Chem* 2001, 276:28509–28515.

34. Hayes CS, Sauer RT. Cleavage of the A site mRNA codon during ribosome pausing provides a mechanism for translational quality control. *Mol Cell* 2003, 12:903–911.

35. Sunohara T, Abo T, Inada T, Aiba H. The C-terminal amino acid sequence of nascent peptide is a major determinant of SsrA tagging at all three stop codons. *RNA* 2002, 8:1416–1427.

36. Sunohara T, Jojima K, Tagami H, Inada T, Aiba H. Ribosome stalling during translation elongation induces cleavage of mRNA being translated in Escherichia coli. *J Biol Chem* 2004, 279:15368–15375.

37. Pedersen K, Zavialov AV, Pavlov MY, Elf J, Gerdes K, Ehrenberg M. The bacterial toxin RelE displays codon-specific cleavage of mRNAs in the ribosomal A site. *Cell* 2003, 112:131–140.

38. Christensen SK, Gerdes K. RelE toxins from bacteria and Archaea cleave mRNAs on translating ribosomes, which are rescued by tmRNA. *Mol Microbiol* 2003, 48:1389–1400.

39. Neubauer C, Gao YG, Andersen KR, Dunham CM, Kelley AC, Hentschel J, Gerdes K, Ramakrishnan V, Brodersen DE. The structural basis for mRNA recognition and cleavage by the ribosome-dependent endonuclease RelE. *Cell* 2009, 139:1084–1095.

40. Baik S, Inoue K, Ouyang M, Nouye M. Significant bias against the ACA triplet in the tmRNA sequence of Escherichia coli K-12. *J Bacteriol* 2009, 191:6157–6166.

41. Garza-Sanchez F, Gin JG, Hayes CS. Amino acid starvation and colicin D treatment induce A-site mRNA cleavage in Escherichia coli. *J Mol Biol* 2008, 378:505–519.

42. Hayes CS, Keiler KC. Beyond ribosome rescue: tmRNA and co-translational processes. *FEBS Lett* 2010, 584:413–419.

43. Holberger LE, Hayes CS. Ribosomal protein S12 and aminoglycoside antibiotics modulate A-site mRNA cleavage and transfer-messenger RNA activity in Escherichia coli. *J Biol Chem* 2009, 284:32188–32200.

44. Cheng K, Ivanova N, Scheres SH, Pavlov MY, Carazo JM, Hebert H, Ehrenberg M, Lindahl M. tmRNA.SmpB complex mimics native aminoacyl-tRNAs in the A site of stalled ribosomes. *J Struct Biol* 2010, 169:342–348.

45. Weis F, Bron P, Rolland JP, Thomas D, Felden B, Gillet R. Accommodation of tmRNA-SmpB into stalled ribosomes: a cryo-EM study. *RNA* 2009, 16:299–306.

46. Shpanchenko OV, Golovin AV, Bugaeva EY, Isaksson LA, Dontsova OA. Structural aspects of trans-translation. *IUBMB Life* 62:120–124.

47. Williams KP, Martindale KA, Bartel DP. Resuming translation on tmRNA: a unique mode of determining a reading frame. *Embo J* 1999, 18:5423–5433.

48. Konno T, Kurita D, Takada K, Muto A, Himeno H. A functional interaction of SmpB with tmRNA for determination of the resuming point of trans-translation. *RNA* 2007, 13:1723–1731.

49. Lee S, Ishii M, Tadaki T, Muto A, Himeno H. Determinants on tmRNA for initiating efficient and precise trans-translation: some mutations upstream of the tag-encoding sequence of Escherichia coli tmRNA shift the initiation point of trans-translation in vitro. *RNA* 2001, 7:999–1012.

50. Miller MR, Healey DW, Robison SG, Dewey JD, Buskirk AR. The role of upstream sequences in selecting the reading frame on tmRNA. *BMC Biol* 2008, 6:29.

51. Watts T, Cazier D, Healey D, Buskirk A. SmpB contributes to reading frame selection in the translation of transfer-messenger RNA. *J Mol Biol* 2009, 391:275–281.

52. Nonin-Lecomte S, Germain-Amiot N, Gillet R, Hallier M, Ponchon L, Dardel F, Felden B. Ribosome hijacking: a role for small protein B during trans-translation. *EMBO Rep* 2009, 10:160–165.

53. Kurita D, Muto A, Himeno H. Role of the C-terminal tail of SmpB in the early stage of trans-translation. *RNA* 2010, 16:980–990.

54. Komarova AV, Tchufistova LS, Supina EV, Boni IV. Protein S1 counteracts the inhibitory effect of the extended Shine-Dalgarno sequence on translation. *RNA* 2002, 8:1137–1147.

55. Herman C, Thevenet D, Bouloc P, Walker GC, D'Ari R. Degradation of carboxy-terminal-tagged cytoplasmic proteins by the Escherichia coli protease HflB (FtsH). *Genes Dev* 1998, 12:1348–1355.

56. Gottesman S, Roche E, Zhou Y, Sauer RT. The ClpXP and ClpAP proteases degrade proteins with carboxy-terminal peptide tails added by the SsrA-tagging system. *Genes Dev* 1998, 12:1338–1347.

57. Choy JS, Aung LL, Karzai AW. Lon protease degrades transfer-messenger RNA-tagged proteins. *J Bacteriol* 2007, 189:6564–6571.

58. Gur E, Sauer RT. Evolution of the ssrA degradation tag in Mycoplasma: specificity switch to a different protease. *Proc Natl Acad Sci USA* 2008, 105:16113–16118.

59. Barends S, Karzai AW, Sauer RT, Wower J, Kraal B. Simultaneous and functional binding of SmpB and EF-Tu-GTP to the alanyl acceptor arm of tmRNA. *J Mol Biol* 2001, 314:9–21.

60. Levchenko I, Seidel M, Sauer RT, Baker TA. A specificity-enhancing factor for the ClpXP degradation machine. *Science* 2000, 289:2354–2356.

61. Flynn JM, Levchenko I, Seidel M, Wickner SH, Sauer RT, Baker TA. Overlapping recognition determinants within the ssrA degradation tag allow modulation of proteolysis. *Proc Natl Acad Sci USA* 2001, 98:10584–10589.

62. Park EY, Song HK. A degradation signal recognition in prokaryotes. *J Synchrot Radiat* 2008, 15:246–249.

63. Song HK, Eck MJ. Structural basis of degradation signal recognition by SspB, a specificity-enhancing factor for the ClpXP proteolytic machine. *Mol Cell* 2003, 12:75–86.

64. Lies M, Maurizi MR. Turnover of endogenous SsrA-tagged proteins mediated by ATP-dependent proteases in Escherichia coli. *J Biol Chem* 2008, 283:22918–22929.

65. Barends S, Zehl M, Bialek S, de Waal E, Traag BA, Willemse J, Jensen ON, Vijgenboom E, van Wezel GP. Transfer-messenger RNA controls the translation of cell-cycle and stress proteins in Streptomyces. *EMBO Rep* 2010, 11:119–125.

66. Milla ME, Brown BM, Sauer RT. P22 Arc repressor: enhanced expression of unstable mutants by addition of polar C-terminal sequences. *Protein Sci* 1993, 2:2198–2205.

67. Withey JH, Friedman DI. The biological roles of trans-translation. *Curr Opin Microbiol* 2002, 5:154–159.

68. Asano K, Kurita D, Takada K, Konno T, Muto A, Himeno H. Competition between trans-translation and termination or elongation of translation. *Nucleic Acids Res* 2005, 33:5544–5552.

69. Zhang Y, Zhang J, Hara H, Kato I, Inouye M. Insights into the mRNA cleavage mechanism by MazF, an mRNA interferase. *J Biol Chem* 2005, 280:3143–3150.

70. Zhang Y, Zhang J, Hoeflich KP, Ikura M, Qing G, Inouye M. MazF cleaves cellular mRNAs specifically at ACA to block protein synthesis in Escherichia coli. *Mol Cell* 2003, 12:913–923.

71. Zhang Y, Zhu L, Zhang J, Inouye M. Characterization of ChpBK, an mRNA interferase from Escherichia coli. *J Biol Chem* 2005, 280:26080–26088.

72. Garza-Sanchez F, Shoji S, Fredrick K, Hayes CS. RNase II is important for A-site mRNA cleavage during ribosome pausing. *Mol Microbiol* 2009, 73:882–897.

73. Abo T, Inada T, Ogawa K, Aiba H. SsrA-mediated tagging and proteolysis of LacI and its role in the regulation of lac operon. *EMBO J* 2000, 19:3762–3769.

74. Retallack DM, Johnson LL, Friedman DI. Role for 10Sa RNA in the growth of lambda-P22 hybrid phage. *J Bacteriol* 1994, 176:2082–2089.

75. Keiler KC, Shapiro L. tmRNA in Caulobacter crescentus is cell cycle regulated by temporally controlled transcription and RNA degradation. *J Bacteriol* 2003, 185:1825–1830.

76. Keiler KC, Shapiro L. TmRNA is required for correct timing of DNA replication in Caulobacter crescentus. *J Bacteriol* 2003, 185:573–580.

77. Quon KC, Yang B, Domian IJ, Shapiro L, Marczynski GT. Negative control of bacterial DNA replication by a cell cycle regulatory protein that binds at the chromosome origin. *Proc Natl Acad Sci USA* 1998, 95:120–125.

78. Wortinger M, Sackett MJ, Brun YV. CtrA mediates a DNA replication checkpoint that prevents cell division in Caulobacter crescentus. *EMBO J* 2000, 19:4503–4512.

79. Domian IJ, Quon KC, Shapiro L. Cell type-specific phosphorylation and proteolysis of a transcriptional regulator controls the G1-to-S transition in a bacterial cell cycle. *Cell* 1997, 90:415–424.

80. Hong SJ, Lessner FH, Mahen EM, Keiler KC. Proteomic identification of tmRNA substrates. *Proc Natl Acad Sci USA* 2007, 104:17128–17133.

81. Cheng L, Keiler KC. Correct timing of dnaA transcription and initiation of DNA replication requires trans translation. *J Bacteriol* 2009, 191:4268–4275.

82. Russell JH, Keiler KC. Subcellular localization of a bacterial regulatory RNA. *Proc Natl Acad Sci USA* 2009, 106:16405–16409.

83. Abe T, Sakaki K, Fujihara A, Ujiie H, Ushida C, Himeno H, Sato T, Muto A. tmRNA-dependent trans-translation is required for sporulation in Bacillus subtilis. *Mol Microbiol* 2008, 69:1491–1498.

84. Stragier P, Kunkel B, Kroos L, Losick R. Chromosomal rearrangement generating a composite gene for a developmental transcription factor. *Science* 1989, 243:507–512.

85. van Wezel GP, McKenzie NL, Nodwell JR. Chapter 5. Applying the genetics of secondary metabolism in model actinomycetes to the discovery of new antibiotics. *Methods Enzymol* 2009, 458:117–141.

86. Hopwood DA. *Streptomyces in Nature and Medicine: The Antibiotic Makers*. New York: Oxford University Press; 2007.

87. Rigali S, Titgemeyer F, Barends S, Mulder S, Thomae AW, Hopwood DA, van Wezel GP. Feast or famine: the global regulator DasR links nutrient stress to antibiotic production by Streptomyces. *EMBO Rep* 2008, 9:670–675.

88. Paget MS, Molle V, Cohen G, Aharonowitz Y, Buttner MJ. Defining the disulphide stress response in Streptomyces coelicolor A3(2): identification of the sigmaR regulon. *Mol Microbiol* 2001, 42:1007–1020.

89. Braud S, Lavire C, Bellier A, Mazodier P. Effect of SsrA (tmRNA) tagging system on translational regulation in Streptomyces. *Arch Microbiol* 2006, 184:343–352.

90. Paleckova P, Bobek J, Felsberg J, Mikulik K. Activity of translation system and abundance of tmRNA during development of Streptomyces aureofaciens producing tetracycline. *Folia Microbiol (Praha)* 2006, 51:517–524.

91. Paleckova P, Felsberg J, Bobek J, Mikulik K. tmRNA abundance in Streptomyces aureofaciens, S. griseus and S. collinus under stress-inducing conditions. *Folia Microbiol (Praha)* 2007, 52:463–470.

92. Yang C, Glover JR. The SmpB-tmRNA tagging system plays important roles in Streptomyces coelicolor growth and development. *PLoS ONE* 2009, 4:e4459.

93. Flardh K, Buttner MJ. Streptomyces morphogenetics: dissecting differentiation in a filamentous bacterium. *Nat Rev Microbiol* 2009, 7:36–49.

94. Bentley SD, Chater KF, Cerdeno-Tarraga AM, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Kieser H, Harper D, et al. Complete genome sequence of the model actinomycete Streptomyces coelicolor A3(2). *Nature* 2002, 417:141–147.

95. Chater KF. Streptomyces inside-out: a new perspective on the bacteria that provide us with antibiotics. *Phil Trans R Soc Lond B Biol Sci* 2006, 361:761–768.

96. Leskiw BK, Bibb MJ, Chater KF. The use of a rare codon specifically during development? *Mol Microbiol* 1991, 5:2861–2867.

97. Rigali S, Nothaft H, Noens EE, Schlicht M, Colson S, Muller M, Joris B, Koerten HK, Hopwood DA, Titgemeyer F, et al. The sugar phosphotransferase system of Streptomyces coelicolor is regulated by the GntR-family regulator DasR and links N-acetylglucosamine metabolism to the control of development. *Mol Microbiol* 2006, 61:1237–1251.

98. van Wezel GP, Woudt LP, Vervenne R, Verdurmen ML, Vijgenboom E, Bosch L. Cloning and sequencing of the tuf genes of Streptomyces coelicolor A3(2). *Biochim Biophys Acta* 1994, 1219:543–547.

99. van Wezel GP, Takano E, Vijgenboom E, Bosch L, Bibb MJ. The tuf3 gene of Streptomyces coelicolor A3(2) encodes an inessential elongation factor Tu that is apparently subject to positive stringent control. *Microbiology* 1995, 141(Pt 10):2519–2528.

100. Bucca G, Brassington AM, Hotchkiss G, Mersinias V, Smith CP. Negative feedback regulation of dnaK, clpB and lon expression by the DnaK chaperone machine in Streptomyces coelicolor, identified by transcriptome and in vivo DnaK-depletion analysis. *Mol Microbiol* 2003, 50:153–166.

101. Wickner S, Hoskins J, McKenney K. Function of DnaJ and DnaK as chaperones in origin-specific DNA binding by RepA. *Nature* 1991, 350:165–167.

102. Kahmann JD, Sass HJ, Allan MG, Seto H, Thompson CJ, Grzesiek S. Structural basis for antibiotic recognition by the TipA class of multidrug-resistance transcriptional regulators. *EMBO J* 2003, 22:1824–1834.

103. Noens EE, Mersinias V, Traag BA, Smith CP, Koerten HK, van Wezel GP. SsgA-like proteins determine the fate of peptidoglycan during sporulation of Streptomyces coelicolor. *Mol Microbiol* 2005, 58:929–944.

104. Noens EE, Mersinias V, Willemse J, Traag BA, Laing E, Chater KF, Smith CP, Koerten HK, van Wezel GP. Loss of the controlled localization of growth stage-specific cell-wall synthesis pleiotropically affects developmental gene expression in an ssgA mutant of Streptomyces coelicolor. *Mol Microbiol* 2007, 64:1244–1259.

105. Traag BA, Kelemen GH, Van Wezel GP. Transcription of the sporulation gene ssgA is activated by the IclR-type regulator SsgR in a whi-independent manner in Streptomyces coelicolor A3(2). *Mol Microbiol* 2004, 53:985–1000.

106. Wang G, Inaoka T, Okamoto S, Ochi K. A novel insertion mutation in Streptomyces coelicolor ribosomal S12 protein results in paromomycin resistance and antibiotic overproduction. *Antimicrob Agents Chemother* 2009, 53:1019–1026.

107. Arsene F, Tomoyasu T, Bukau B. The heat shock response of Escherichia coli. *Int J Food Microbiol* 2000, 55:3–9.

108. Musatovova O, Dhandayuthapani S, Baseman JB. Transcriptional heat shock response in the smallest known self-replicating cell, Mycoplasma genitalium. *J Bacteriol* 2006, 188:2845–2855.

109. Manteca A, Mader U, Connolly BA, Sanchez J. A proteomic analysis of Streptomyces coelicolor programmed cell death. *Proteomics* 2006, 6:6008–6022.

110. Ranquet C, Gottesman S. Translational regulation of the Escherichia coli stress factor RpoS: a role for SsrA and Lon. *J Bacteriol* 2007, 189:4872–4879.

111. Fredriksson A, Ballesteros M, Peterson CN, Persson O, Silhavy TJ, Nystrom T. Decline in ribosomal fidelity contributes to the accumulation and stabilization of the master stress response regulator

sigmaS upon carbon starvation. *Genes Dev* 2007, 21:862–874.

112. Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, Romero DA, Horvath P. CRISPR provides acquired resistance against viruses in prokaryotes. *Science* 2007, 315:1709–1712.

113. Brouns SJ, Jore MM, Lundgren M, Westra ER, Slijkhuis RJ, Snijders AP, Dickman MJ, Makarova KS, Koonin EV, van der Oost J. Small CRISPR RNAs guide antiviral defense in prokaryotes. *Science* 2008, 321:960–964.

114. van der Oost J, Brouns SJ. RNAi: prokaryotes get in on the act. *Cell* 2009, 139:863–865.

115. Montero CI, Lewis DL, Johnson MR, Conners SB, Nance EA, Nichols JD, Kelly RM. Colocation of genes encoding a tRNA-mRNA hybrid and a putative signaling peptide on complementary strands in the genome of the hyperthermophilic bacterium Thermotoga maritima. *J Bacteriol* 2006, 188:6802–6807.

116. Dulebohn D, Choy J, Sundermeier T, Okan N, Karzai AW. Trans-translation: the tmRNA-mediated surveillance mechanism for ribosome rescue, directed protein degradation, and nonstop mRNA decay. *Biochemistry* 2007, 46:4681–4693.

117. Karzai AW, Roche ED, Sauer RT. The SsrA-SmpB system for protein tagging, directed degradation and ribosome rescue. *Nat Struct Biol* 2000, 7:449–455.

118. Moore SD, Sauer RT. The tmRNA system for translational surveillance and ribosome rescue. *Annu Rev Biochem* 2007, 76:101–124.

119. Withey JH, Friedman DI. A salvage pathway for protein structures: tmRNA and trans-translation. *Annu Rev Microbiol* 2003, 57:101–123.

FURTHER READING

Excellent additional papers and reviews that have not been mentioned in the text are among others: Duhlebohn et al.[116]; Karzai et al.[117]; Moore and Sauer[118] and Withey and Friedman.[119]

For detailed information on tmRNA, including all known sequences, we refer to the two very comprehensive tmRNA databases maintained by Jacek Wower at Auburn University (http://www.ag.auburn.edu/mirror/tmRDB/) and by kelly Williams at Indiana University (http://www.indiana.edu/~tmrna/).