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Quality Control of Bacterial mRNA Decoding and Decay

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Abstract

Studies in eukaryotes and prokaryotes have revealed that gene expression is not only controlled through altering the rate of transcription but also through varying rates of translation and mRNA decay. Indeed, the expression level of a protein is strongly affected by the steady state level of its mRNA. RNA decay can, along with transcription, play an important role in regulating gene expression by fine-tuning the steady state level of a given transcript and affecting its subsequent decoding during translation. Alterations in mRNA stability can in turn have dramatic effects on cell physiology and as a consequence the fitness and survival of the organism. Recent evidence suggests that mRNA decay can be regulated in response to environmental cues in order to enable the organism to adapt to its changing surroundings. Bacteria have evolved unique post transcriptional control mechanisms to enact such adaptive responses through: 1) general mRNA decay, 2) differential mRNA degradation using small non-coding RNAs (sRNAs), and 3) selective mRNA degradation using the tmRNA quality control system. Here, we review our current understanding of these molecular mechanisms, gleaned primarily from studies of the model gram negative organism *E. coli*, that regulate the stability and degradation of normal and defective transcripts.

Keywords

RNA quality control; SmpB; tmRNA; *trans*-translation; RNase R; RNA decay

1. Introduction

As with other biomolecules, the great variety of RNA species produced by the bacterial cell require quality control mechanisms to ensure proper folding and function. In addition, the role of mRNA as a template for protein synthesis adds greater significance to mRNA quality control. The translation of a faulty transcript without adequate quality assurance measures might lead to the accumulation of aberrant protein products that could be detrimental to the cell. While much data have been generated recently on the mechanisms of eukaryotic mRNA quality control, especially on that of nonsense mediated decay (NMD, reviewed elsewhere in this issue), the related topics in prokaryotes are comparatively less explored. Generally, bacterial mRNAs are not post-transcriptionally spliced, nor do they exhibit the 5'-cap structures of their eukaryotic counterparts. As such, post-transcriptional quality control processes of prokaryotic mRNA are distinct from the corresponding processes in eukaryotes. This review focuses on

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issues related to post-transcriptional processing, targeting, and degradation of bacterial mRNAs including those facilitated by small regulatory RNAs, with special emphasis on tmRNA and *trans*-translation.

2. Bacterial mRNA decay

A comparison of the stability of bacterial and eukaryotic mRNAs reveals that bacterial mRNAs have a comparatively brief existence. The half-lives of most bacterial mRNAs range from 40 seconds to 60 minutes, whereas the half-lives of some eukaryotic mRNAs can be as long as several days [1]. Variation in the stability of transcripts has an important role in the control of protein expression within the cell, as long-lived transcripts are generally subject to more rounds of translation than those with a shorter half-life. Several factors play a role in controlling the lifespan of specific mRNAs by regulating their propensity to be degraded. Although the detailed mechanism of prokaryotic mRNA decay remains to be fully elucidated, both 5'-and 3'-end dependent degradation pathways have been described [2]. The rate of mRNA turnover, the nucleases involved, and the directionality of degradation seem all to depend on the bacterial species, the rate of translation of the mRNA, as well as sequence and structural elements present within the transcript [3–6].

2.1. 5'-terminus dependant endonucleases

One general pathway for prokaryotic mRNA decay, using *E. coli* as a model system, has been well characterized. It begins with an endonucleolytic cleavage at one or more defined sites within the transcript in a 5'-end dependent manner. The 5'-terminus of the mRNA appears to be of particular importance in initiating the decay process. Yet, *E. coli* lacks any processive 5'-3'-exoribonucleases [7] – although a 5'-3'-exoribonuclease activity has been reported in *Bacillus subtilis* [4]. The most commonly cited experiments that demonstrate the importance of the 5'-end in *E. coli* mRNA decay are those concerning the *ompA* transcript. The *ompA* transcript has a long half-life (for an *E. coli* mRNA) of 15–20 minutes [8] that is dependent on its 133-nt 5'-untranslated region (5'-UTR). Fusion of the *ompA* 5'-UTR sequence to the 5'-end of a heterologous mRNA lengthens the half-life of the mRNA [9,10]. Further investigation of this effect has revealed that a stem-loop structure at the 5'-end of the mRNA, a high degree of ribosomal occupancy near the initiation codon, and the presence of ribosomes translating the protein coding region are all factors that affect the rate of endonucleolytic cleavage of the mRNA, slowing its rate of decay [8]. Interestingly, addition of a region of unpaired, single stranded nucleotides prior to the stem-loop structure reverses the mRNA stabilization effect of the *ompA* 5′-UTR [11]. Based on these and related studies a model has been proposed whereby 5'-end dependant decay of primary transcripts in *E. coli* is initiated by an endoribonuclease that requires a single stranded 5'-landing site to gain access to internal cleavage sites.

Of the known *E. coli* endoribonucleases, RNase E is thought to be the principle nuclease responsible for this cleavage [12]. In the absence of RNase E activity there is stabilization of a wide range of mRNAs [13–15], which supports the idea that RNase E is the main endoribonuclease in this process. However, other endonucleases also participate in initiating mRNA decay. The endonucleases that have been implicated in these alternate pathways are perhaps more specialized and include RNase III, which cleaves double stranded RNAs [16], RNase G, which has similar specificity to RNase E but is responsible for far fewer cleavages *in vivo* [17,18], and RNase Z, which might have a more prominent role in organisms that lack RNase E function [19]. Although RNase E can act alone, it is part of a multiprotein complex called the degradosome and is thought to function in cooperation with these factors (see [20] and references therein). In addition to RNase E, the degradosome also contains polynucleotide phosphorylase (PNPase), an ATP-dependent helicase (RhlB) and enolase [20].

From a mechanistic perspective, RNase E requires 5’ single-stranded RNA of at least four nucleotides in length for efficient binding and degradation. RNase E also prefers 5’-monophosphorylated substrates for full activity [21], as natural substrates with terminal 5’-triphosphates are poorly cleaved [22]. The slow decay rate of 5’-triphosphorylated substrates led to the question of how mRNA decay is initiated by RNase E if its preferred substrates are monophosphorylated mRNA transcripts. Recent work has shown that there may be a rate limiting conversion prior to RNase E endonucleolytic cleavage, whereby the triphosphorylated 5’-terminus is converted to a monophosphorylated form [23]. It was recently demonstrated that the RppH protein, a pyrophosphohydrolase enzyme, catalyzes the 5’-pyrophosphate removal reaction [24]. In light of these findings, mRNA decay is initiated by a 5’-pyrophosphate removal step allowing an initial, internal endonucleolytic cleavage by RNase E – provided the 5’-terminus is available and not sequestered in a hairpin structure. This first internal cleavage then presents the substrates for a series of endo- and exo-nucleolytic reactions, rapidly reducing the mRNA to mononucleotides (Figure 1). The preferential decay of monophosphorylated substrates explains the observation that complete mRNA decay in *E. coli* occurs very rapidly after an initial 5’-end dependant endonucleolytic cleavage event. Rapid decay occurs due to the monophosphorylated 5’-end of the 3’-cleavage fragments, making these RNAs ideal substrates for RNase E. Furthermore, RNase E and the 3′-5′ exonucleases act cooperatively to rapidly degrade the cleaved transcript to mononucleotides. Furthermore, the cleavage dependent loss of the 5’-UTR and ribosome-binding site inhibits translation initiation and removes the shielding effect of translating ribosomes on the mRNA.

2.2. 3’-terminus and 3’-to-5’ exonucleases

mRNA decay in *E. coli* comprises a number of parallel pathways utilizing endoribonucleases and exoribonucleases, both on their own and in combination. Of the exoribonucleases present in *E. coli*, two enzymes, RNase II and PNPase, are thought to perform the bulk of 3′-to-5′ exoribonucleolytic decay. Both enzymes degrade the mRNA from the 3’-terminus, one nucleotide at a time, resulting in individual mononucleotides and a 5’-end oligonucleotide remnant of 2 to 5 nucleotides. The short oligonucleotide products of RNase II and PNPase action are converted to mono-nucleotides through the activity of oligoribonuclease (Fig. 1 and [25]). There are differing reports of the share of exonucleolytic decay performed by RNase II and PNPase. Recent studies suggest that although both RNases play prominent roles in mRNA degradation, PNPase may be a more significant contributor to the decay process [26,27]. The ability to deal with RNA secondary structures is a known difference between RNase II and PNPase. RNase II can efficiently degrade single-stranded RNA but is incapable of unwinding secondary structures. PNPase is known to favor single stranded substrates and pause at stem-loop structures. Both enzymes are unable to bind substrates with fewer than 6–10 unpaired bases at the 3’-end [28]. PNPase, however, has been shown to have some ability to degrade secondary structural elements [29]. PNPase degradation of structured mRNA elements is thought to be aided via polyadenylation by poly (A) polymerase I (PAP I). PNPase might also require assistance from the RhlB helicase for particularly stable structures.

Polyadenylation of the 3’-end of a bacterial RNA, in contrast to eukaryotic mRNAs, has been shown to have a role in facilitating decay. Recent evidence suggests that endonucleolytic cleavage by RNase E provides the signals required for polyadenylation of the resultant fragments. This might be facilitated by direct targeting of PAP I to these fragments through its interaction with the RNase E degradosome [30]. It is suggested that addition of a single stranded 3’- poly-A tail to an mRNA promotes binding of PNPase. Upon binding to the poly-A tail, PNPase may then degrade the RNA until it reaches the structured region, whereupon it stalls. Stalled PNPase can then resume through the structured region if there is a temporary unwinding of the stem structure, possibly facilitated by the action of the RhlB helicase. Recent studies suggest that polyadenylation plays a much more pivotal role in bacterial RNA quality control

than previously anticipated. The significance and extent of polyadenylation was highlighted by the finding that >90 % of transcripts in *E. coli* are modified by PAP I at some stage of their life cycle [31].

2.3. Role of stem-loop structures in regulating mRNA decay rates

The presence of a stem-loop in an mRNA can perform a regulatory function that influences the expression of particular genes by lengthening the half-lives of their transcripts. Bacterial mRNAs frequently possess a stem loop structure at their 3'-end. The slow rate at which 3'-5' exoribonucleases can degrade such structures provides some degree of protection from decay. Indeed, the absence of a 3'-end structure, which can be brought about by an endonucleolytic cleavage, results in very rapid RNA decay [1]. Differential expression of ORFs present on polycistronic transcripts is also achieved via the inability of bacterial exoribonucleases to degrade highly structured Repeated Extragenic Palindromic (REP) elements. The presence of REP elements in a polycistronic message can protect upstream sequences from decay, lengthening their lifetimes relative to other downstream sequences [1].

3'-5' exonucleolytic decay proceeds up to a stem loop, leaving shorter decay intermediates with a stem loop at the 3'-end. The degradation of these small intermediates is a critical step in the decay pathway. Recent work suggests that the decay of these REP stabilizers involves not just PAP I and PNPase, but also RhlB and the degradosome [32]. In particular, RhlB and RNase E, both components of the degradosome, have been shown to be necessary for REP-stabilizer degradation. One interesting question is how the bacterial mRNA decay machinery differentiates between primary transcripts and endonucleolytic cleavage products. A stem-loop at the 3'-end of a primary transcript protects it from exonucleolytic decay, yet the 3'-end of a 5'-fragment generated by endonucleolytic cleavage is more rapidly degraded. This degradation not only occurs through single stranded regions but also through structured regions, via the combined actions of RhlB, poly (A) polymerase and PNPase. In addition, studies have established that poly (A) polymerase activity in degradation of stem-loop regions is initiated by an RNase E-mediated cleavage event [33].

Recently an additional 3'-5' exonuclease, RNase R, has been implicated in the decay of REP-stabilizers. RNase R is a hydrolytic 3'-5' exonuclease with considerable sequence homology to RNase II. Yet unlike RNase II it has the ability to degrade through double stranded regions of RNA, including REP-stabilizers [34]. As with RNase II and PNPase, RNase R requires a single stranded 3'-end to bind and initiate decay. However, upon binding and initiation of exonucleolytic decay, RNase R proceeds much more efficiently through secondary structural elements within the RNA. Although RNase R is widely conserved in eubacteria and may conceivably play a critical role in RNA decay, little is known about its mode of action. Intriguingly, RNase R is induced under stress conditions in *E. coli* [35] and is associated with the degradosome under cold shock conditions in *Pseudomonas syringae* [36], indicating a role for the enzyme in mRNA decay under stress conditions. Consistent with this conclusion, Purusharth and colleagues recently reported that RNase R is essential for growth of *Pseudomonas syringae* at low temperature, and that under these conditions *rnr* mutants accumulate aberrant 5S and 16S rRNA variants exhibiting 3'-end processing defects [37]. These results suggest that RNase R may have a specialized role in 3'-5' exonucleolytic decay that neither RNase II nor PNPase can fulfill.

3. Small non-coding RNA regulators in mRNA decay

Since the discovery of the phenomenon of RNA silencing in eukaryotes, small non-protein coding RNA (sRNA) regulators have become the subject of an explosively growing field of investigation. In comparison to eukaryotic small RNAs, our knowledge of similar types of regulators in prokaryotes is more limited. Nevertheless, sRNA regulators of mRNA stability

and expression are present in prokaryotes and participate in a variety of key cellular functions including: adaptation to environmental and nutritional stresses, quorum sensing, virulence factor production, and plasmid maintenance [38–40].

A large number of sRNAs act by directly base pairing, albeit imperfectly, with short sequences in the 5'-UTR of target mRNAs. The known outcomes of such pairing include both repression of translation, coupled with rapid degradation of the target mRNA, as well as activation of translation, coupled with stabilization of the target mRNA. In most known cases of repression, sRNAs prevent initiation of translation by binding in the vicinity of the ribosome-binding site of the target mRNAs and blocking ribosomal access and translation initiation (Fig. 2). For instance, the well-characterized *E. coli* sRNAs OxyS, MicA, and MicC have been shown to directly interfere with 30S ribosome binding of target mRNAs [41,42]. RyhB, the sRNA regulator of iron metabolism, which targets transcripts encoding various iron-binding proteins, is thought to function in this manner [40]. RyhB is induced under iron-depleted conditions, binds in the vicinity of the ribosome-binding site of its target mRNA and facilitates its selective degradation [43,44]. It is likely that other known *E. coli* sRNAs, such as DsrA, MicF, SgrS, and Spot42, use a similar mechanism to affect the translation and decay of their target transcripts [45–47].

Curiously, the stability and target binding activities of RyhB, and many other sRNAs, are in large part dependent on the presence of a ubiquitous and highly abundant small RNA chaperone Hfq [40,42,48–54]. Hfq belongs to a family of Sm-like proteins, which in eukaryotes take part in mRNA splicing. Hexameric Hfq binds with high affinity to A/U rich segments of sRNAs to promote efficient pairing with target mRNAs [49–51,53–57]. Formation of the sRNA-mRNA complex not only inhibits translation of the target transcript, but also facilitates the degradation of both the target mRNA and sRNA [57,58]. The degradation process is initiated by the endonucleolytic activity of either RNase III or RNase E. While RNase III can directly recognize the sRNA-mRNA duplex, it is not clear how RNase E is targeted to this complex (Fig. 2B). One possible mechanism is that the sRNA-mediated translation block unmasks RNase E sensitive sites that are usually protected by the translating ribosomes [59]. Another possible mechanism might involve changes in the mRNA secondary structure, induced by sRNA pairing, that make the target mRNA more susceptible to recognition and cleavage by RNase E. Several recent experiments suggest that RNase E is recruited to the sRNA regulatory complex via direct interaction with sRNA chaperone Hfq [48,57,60,61]. Therefore, bacterial small regulatory RNAs perform an important mRNA quality control function by selectively removing transcripts whose encoded protein products are no longer needed in the cell.

4. tmRNA and translation quality control

A small bacterial RNA that has received a great deal of attention is transfer messenger RNA (tmRNA). tmRNA along with its requisite protein partner Small protein B (SmpB) orchestrates an elegant translational control process termed *trans*-translation [62–71] Gene mutation, DNA damage, mRNA damage, and translational errors may all lead to ribosomes reaching the 3'-end of an mRNA without encountering an in-frame termination codon. This event could have two potentially hazardous consequences for the bacterium (Fig. 3A). First, since an in-frame stop codon is required to recruit the translation termination apparatus, mRNAs lacking in-frame stop codons lead to ribosome stalling and significant loss of translational efficiency. Secondly, aberrant protein products translated from incomplete mRNAs may be harmful to cells. The SmpB•tmRNA quality control system solves both problems by recognizing and rescuing stalled ribosomes, and directing the addition of a C-terminal proteolysis tag to incomplete protein products [64,66,71–79] (Fig. 3B). In addition, the SmpB•tmRNA system targets the aberrant mRNA for degradation, thus preventing future ribosome stalling events [62,67,69].

4.1. tmRNA and SmpB

tmRNA is a unique bi-functional RNA molecule that exhibits features and activities similar to both tRNA and mRNA. The 5'-and 3'-ends fold to form a tRNA-like domain with sequence and structural similarity to $tRNA^{Ala}$ [80–84]. The tRNA-like domain of tmRNA possesses an amino acid acceptor stem, a TΦC arm and a D-loop, but lacks an anticodon arm. Like $tRNA^{Ala}$, tmRNA can be charged with alanine through the action of alanyl-tRNA synthetase (Ala-RS) [84,85]. tmRNA exhibits additional secondary structural elements in the form of four RNA pseudoknots (pk1-pk4). The specific functions of the tmRNA pseudoknots remain unclear, however several studies suggest that pk1 is important for tmRNA structure and possibly function [85–90]. Between pseudoknots 1 and 2 in the tmRNA sequence lies the mRNA-like open reading frame (ORF), which codes for a proteolytic degradation tag (ANDENYALAA in *E. coli*) followed by tandom UAA termination codons.

The essential protein partner of tmRNA, SmpB, is a small basic RNA binding protein that binds tmRNA with high affinity and specificity [73,91]. SmpB is required for stable interactions of tmRNA with stalled ribosomes [73,92]. The structure of SmpB protein has been solved by both NMR and X-ray crystallography [75,93–95]. It has an antiparallel β-barrell type structure with an embedded oligonucleotide binding (OB) fold [75,93–95]. The tmRNA binding residues of SmpB appear to be clustered on a unique surface of the protein [75,91, 93]. Alterations that affect this surface of the protein abolish tmRNA binding acitivity both *in vivo* and *in vitro* [91]. SmpB also possesses a C-terminal tail that is unstructured in solution. However, the C-terminal tail is functionally indespensible for *trans*-translation and might gain structure upon ribosome binding [96,97]. The C-terminal tail of SmpB seems to be involved in facilitating the tRNA-like activity of tmRNA, perhaps acting as an anti-codon arm mimic [62,97]. SmpB variants that lack the C-terminal tail, or harboring specific mutations within this region, do not support *trans*-translation – despite being fully capable of binding tmRNA and delivering it to stalled ribosomes [97].

4.2. The mechanism of *trans*-translation

Trans-translation begins with recognition of stalled ribosomes by SmpB and tmRNA. The tRNA-like domain of tmRNA is charged with alanine by Ala-RS. The GTP bound form of elongation factor Tu (EF-Tu-GTP) recognizes alanylated-tmRNA, and a quaternary complex of SmpB•tmRNA•EF-Tu(GTP) binds in the A-site of stalled ribosomes. GTP is hydrolyzed, EF-Tu(GDP) is released, and the tRNA-like domain of tmRNA is accommodated into the ribosomal A-site. The growing polypeptide is then transferred onto the tRNA-like domain of tmRNA. Next, the stalled ribosome is promoted to disengage from the defecive mRNA and engage the mRNA-like sequence of tmRNA as a surrogate template. The tmRNA encoded ORF is translated normally until the ribosome reaches a termination codon at the end of the tmRNA reading frame. This allows for rapid translation termination, protein release, and ribosome recycling. The nascent polypeptide, now carrying an 11 amino acid C-terminal degradation tag, is efficiently recognized and degraded by cellular proteases. Several cellular proteases, including ClpXP, ClpAP, Lon, FtsH, and Tsp have been shown to be involved in degradation of tmRNA tagged proteins [63,66,98–100]. The SmpB•tmRNA system also facilitates the selective degradation of the aberrant mRNA by RNase R [67,69,101,102] (see below).

4.3. SmpB•tmRNA substrates

What differentiates a stalled ribosome, making it a substrate for SmpB•tmRNA binding, remains an open question. A variety of situations which delay the progress of protein synthesis have been shown to elicit tagging by the SmpB•tmRNA system. Tagging can occur at the end of a non-stop mRNA, but also at internal positions on the mRNA [66,103–105]. However, it is likely that in cases of internal tagging, a co-translational mRNA cleavage event leads to the

generation of a non-stop mRNA [101,106–109]. As such, one possible mechanism for the differentiation between normal and stalled ribosomes is the presence of mRNA sequence within or 3'-to the ribosomal A-site. Tagging has also been reported at positions corresponding to weak termination codons, presumably due to slow rates of release factor binding to the A-site, as compared to the rate of cognate tRNA A-site entry [105,109–111]. Indeed, depletion of the cognate release factor has been shown to enhance the rate of tagging at a specific termination codon [112,113]. In addition, tRNA scarcity can lead to SmpB•tmRNA-mediated tagging [67,69,104,108]. This observation is highlighted by the discovery that overexpression of an mRNA containing tandem rare arginine codons leads to tagging, an effect that is rescued by driving increased expression of the cognate tRNA. Tagging has also been observed in response to programmed ribosome stalling events such as translation of the secM arrest sequence [114,115]. Our current understanding is that various stress conditions could lead to unproductively stalled ribosomes. Such stalled ribosomes are not immediately a substrate for the SmpB•tmRNA mediated *trans*-translation process. They become a substrate only after an as yet unknown endonuclease cleaves the causitive mRNA within the A-site of the ribosme, freeing the A-site decodong center for efficient SmpB•tmRNA binding to commence *trans*-translation [62,69,107,113].

4.4. Decay of defective mRNAs

In addition to the ribosome rescue and directed proteolysis activities in *trans*-translation, recent work has shown that tmRNA promotes the decay of mRNA transcripts that lead to ribosome stalling. Yamamoto *et al.* [102] were the first to report a role of tmRNA in mRNA decay. They utilized a non-stop *crp* mRNA reporter gene that was shown to engage the *trans*-translation machinery. They found that this non-stop transcript was rapidly degraded in cells containing tmRNA, while in cells lacking tmRNA it accumulated to higher steady state levels. This finding supported a role for tmRNA in facilitating the degradation of non-stop mRNAs.

Work in our laboratory has confirmed the role of *trans*-translation in promoting the degradation of faulty mRNAs that cause ribosome stalling [67,69]. Interestingly, we have also found that specific sequence elements within tmRNA are required to facilitate the decay of non-stop transcripts engaged in *trans*-translation. Mehta *et al.* [67] demonstrated that certain nucleotides within the last three codons of the tmRNA encoded reading frame are required for efficient and selective decay of defective mRNAs. tmRNA variants carrying substitutions of these nucleotides that alter neither the secondary structure of the mRNA-like domain nor the amino acid sequence of the proteolysis tag were found to be defective in facilitating mRNA decay. Importantly, these mRNA decay-defective tmRNA variants showed similar expression levels and stability to wild type tmRNA. In addition, these tmRNA variants were fully competent in supporting the ribosome rescue and reporter protein tagging functions of tmRNA.

Furthermore, we have found that tmRNA-mediated mRNA decay requires the processive 3'-5' exoribonuclease RNase R. RNase R has previously been shown to associate with a ribonucleoprotein complex consisting of ribosomal protein S1, phosphoribosyl pyrophosphate synthase, tmRNA and SmpB [116]. To test the putative involvement of processive 3'-5' exoribonucleases, we made use of *in vivo* mRNA decay assays involving both non-stop and rare codon containing reporter mRNAs. We showed that RNase R, tmRNA and SmpB were all required for rapid decay of both non-stop and rare codon containing mRNAs [69]. In contrast, the steady state level of a related normal mRNA reporter (with a stop codon and no rare codons) was not effected by the presence or absence of tmRNA or RNase R [69]. These results suggested that a functional *trans*-translation system and RNase R are both required for the selective disposal of aberrant mRNAs that promote ribosome stalling. Three possible mechanisms were proposed for tmRNA-mediated decay of defective mRNAs. The first is that prior to disengagement of the mRNA from the rescued ribosome, interactions of certain

nucleotides in the stop codon proximal segment of the tmRNA ORF with ribosomal elements promote binding, rearrangement or loading of RNase R onto the defective mRNA. The second possible mechanism involves the tmRNA ORF region interacting with other elements of tmRNA or other tmRNA-associated factors that then facilitate decay of the faulty mRNA by RNase R. The third explanation is that this region of the tmRNA reading frame interacts directly with RNase R to bring it to the 3'-terminus of the faulty mRNA and thus promote exonucleolytic decay of the transcript.

Several additional *trans*-translation related roles of RNase R have been reported. Cairrao *et al.* [117] have indicated that RNase R is required for the processing of the pre-tmRNA transcript to generate functional tmRNA under cold shock conditions. Furthermore, Hong *et al.* [118] analyzed degradation of a two piece *Caulobacter crescentus* tmRNA and have suggested that RNase R is required for the cell cycle dependent degradation of tmRNA. SmpB is thought to protect tmRNA from degradation by RNase R. As the levels of SmpB protein decrease at specific stages of *Caulobacter crescentus* cell cycle, RNase R gains access to the SmpB-protected 3'-ends of this two piece tmRNA to enact its degradation [118]. Therefore, the interplay between SmpB and RNase R is thought to regulate the cell cycle dependent stability and activity of *Caulobacter crescentus* tmRNA.

4.5. Physiological significance of the SmpB•tmRNA system

The genes encoding SmpB protein and tmRNA are represented in all sequenced bacterial genomes. The *ssrA* gene (encoding tmRNA) is not essential under ideal growth conditions in *E. coli*. *ssrA* deficient strains, however, exhibit slower growth at high temperature, slow recovery from carbon starvation, and reduced motility [84,119]. Similarly, *ssrA* is not required for normal growth of *Bacillus subtilis*, however *smpB* and *ssrA* deletions lead to reduced growth rate at both low and high temperatures [120]. The SmpB•tmRNA translational quality control system is important for survival and pathogenesis of some bacterial species. Genome wide mutagenesis results suggest that the *ssrA* and *smpB* genes are essential in both *Mycoplasma genitalium* and *Mycoplasma pneumoniae* [121]. The *ssrA* gene is also thought to be essential for survival in *Neisseria gonorrhoeae* [122]. Although non-essential, *smpB* and *ssrA* are important for virulence in *Salmonella enterica* [123,124], and *Yersinia pseudotuberculosis* [124]. Interestingly, in *Yersinia pseudotuberculosis*, loss of SmpB and tmRNA leads to severe defects in the expression and delivery of virulence effector proteins [124]. It is likely that this effect contributes to the loss of virulence observed in these mutants. Hence, the SmpB•tmRNA system is a prokaryotic-specific quality control mechanism that is important for survival and virulence of some pathogenic bacteria.

SmpB•tmRNA mediated tagging is also thought to serve as a mechanism for translational control of gene expression under normal growth conditions. A number of regulatory proteins have been shown to be tagged by the SmpB•tmRNA system, for example YbeL, GalE, RbsK, and the LacI repressor are all substrates of the SmpB•tmRNA system [105,125]. In the case of LacI, it is thought that tagging of the repressor serves as a negative feedback mechanism to maintain optimal repressor concentrations. Consistent with this hypothesis, *ssrA*$^{-}$ cells exhibit a delay in induction of the lac operon [105,125]. More recently, tmRNA has been implicated in the maintenance of cellular concentration of the *E. coli* stress sigma factor RpoS [126]. RpoS is upregulated in response to various cellular stresses. The stationary phase upregulation of RpoS is substantially reduced in the absence of tmRNA, and the effect appears to be at the level of RpoS translation [126].

4.6. Comparison of prokaryotic and eukaryotic translation surveillance mechanisms

Unproductive ribosome stalling is a very basic cellular event with a variety of causes and consequences. However, prokaryotic and eukaryotic cells deal with the phenomenon in distinct

ways. Bacterial *trans*-translation, in comparison to eukaryotic mechanisms, is a comparitivly robust response to ribosome stalling, as non-stop mRNAs, rare codon containing mRNAs, as well as mRNAs with specific stall sequences are all targets for the SmpB•tmRNA system [66,103,104,115]. In contrast, eukaryotic cells exhibit at least two distinct mechanisms for preventing ribosome stalling. The eukaryotic non-stop mRNA decay (NSD) pathway targets transcripts lacking in-frame termination codons [127–132], while the no-go decay (NGD) pathway deals with mRNAs containing internal stall sites (i.e., mRNAs with secondary structural elements such as stem loops and pseudoknots) [133]. NSD involves exosome mediated 3′-5′ mRNA decay, mediated by the C-terminal domain of Ski7p [127,131] – although a 5′-3′ non-stop mRNA decay activity has been reported [128]. No-go decay is initiated by an mRNA cleavage event performed by an as yet unidentified nuclease and involves two proteins, Dom34p and Hbs1p, which exhibit similarity to the eukaryotic release factor eRF-1 [133]. Translation inhibition as well as nascent protein destabilization are thought to limit the accumulation of protein products translated from non-stop mRNAs [128–130,132], but the fate of nascent polypeptides is unclear in no-go decay. It has been convincingly demonstrated that non-stop mRNAs lead to translational repression, as non-stop mRNA reporters accumulate in polysome fractions [128]. However, the mechanism of ribosome rescue and restoration of translational efficiency is unclear. Similarly, secondary structure elements present in mRNAs that elicit no-go decay must result in sequestration of ribosomes and it is, as yet, unclear how this unproductive stalling is alleviated.

Trans-translation is an elegant, simple and comprehensive mechanism for dealing with unproductive ribosome stalling. It can efficiently deal with different types of stalling events, including non-stop mRNAs, multiple rare codons, and engineered stall sequences. In doing this, the SmpB•tmRNA system promotes ribosome rescue, directs the degradation of aberrant protein products, and facilitates the decay of the causative non-stop mRNA (Fig. 3B). Considering the versatility and efficiency of this system, it seems surprising that such a complete mechanism for dealing with a host of universal cellular problems has been lost in eukaryotes. One possible explanation is that eukaryotes have evolved more efficient mechanisms for the individual activities performed by the SmpB•tmRNA system. Another possibility is that a *trans*-translation-like mechanism would interfere with productive ribosome stalling events. For instance, SmpB•tmRNA might interfere with translational control processes such as programmed ribosomal frameshifting or delayed translation to facilitate proper co-translational folding of nascent proteins. More details about the way eukaryotic cells deal with unproductive ribosome stalling events may help shed light on this issue.

5. Conclusions and Perspectives

The unique properties of bacterial mRNAs necessitate distinct prokaryotic mRNA decay and quality control mechanisms. We have discussed progress in the complex field of bacterial mRNA decay, and have shown that controlling the rate of transcript degradation represents a mechanism for control of gene expression. Bacterial mRNA decay involves 5’- and 3’-end dependent mechanisms facilitated by several exo- and endo-ribonucleases. Sequence and structural elements within the transcript regulate both its rate of decay and the primary nucleases involved. We also discussed the role of small non-coding RNAs in post-transcriptional gene regulation and described models for how these regulatory RNAs contribute to mRNA quality control and stability. Finally, we discussed the SmpB•tmRNA-mediated mechanism for translation quality control and selective mRNA decay. These mechanisms contribute both to providing additional routes for post-transcriptional control of gene expression and relieving cellular stresses caused by aberrant transcripts.

Although there has been rapid progress in our understanding of the role of ribonucleases in post-transcriptional control of mRNA stability and decay, clearly a tremendous amount is yet

to be learned. It is also abundantly clear that a thorough understanding of the post-transcriptional regulation of gene expression will require studies not only in *E. coli* but also in other related and distant bacterial species. The recent discovery of 5'-to-3' exonucleases in *Bacillus subtilis*, the RNase activity and the substrate range of the toxin–antitoxin systems, and the ribozyme-like activity of certain riboswitches highlight only a few examples of other novel mechanism that remain to be elucidated.

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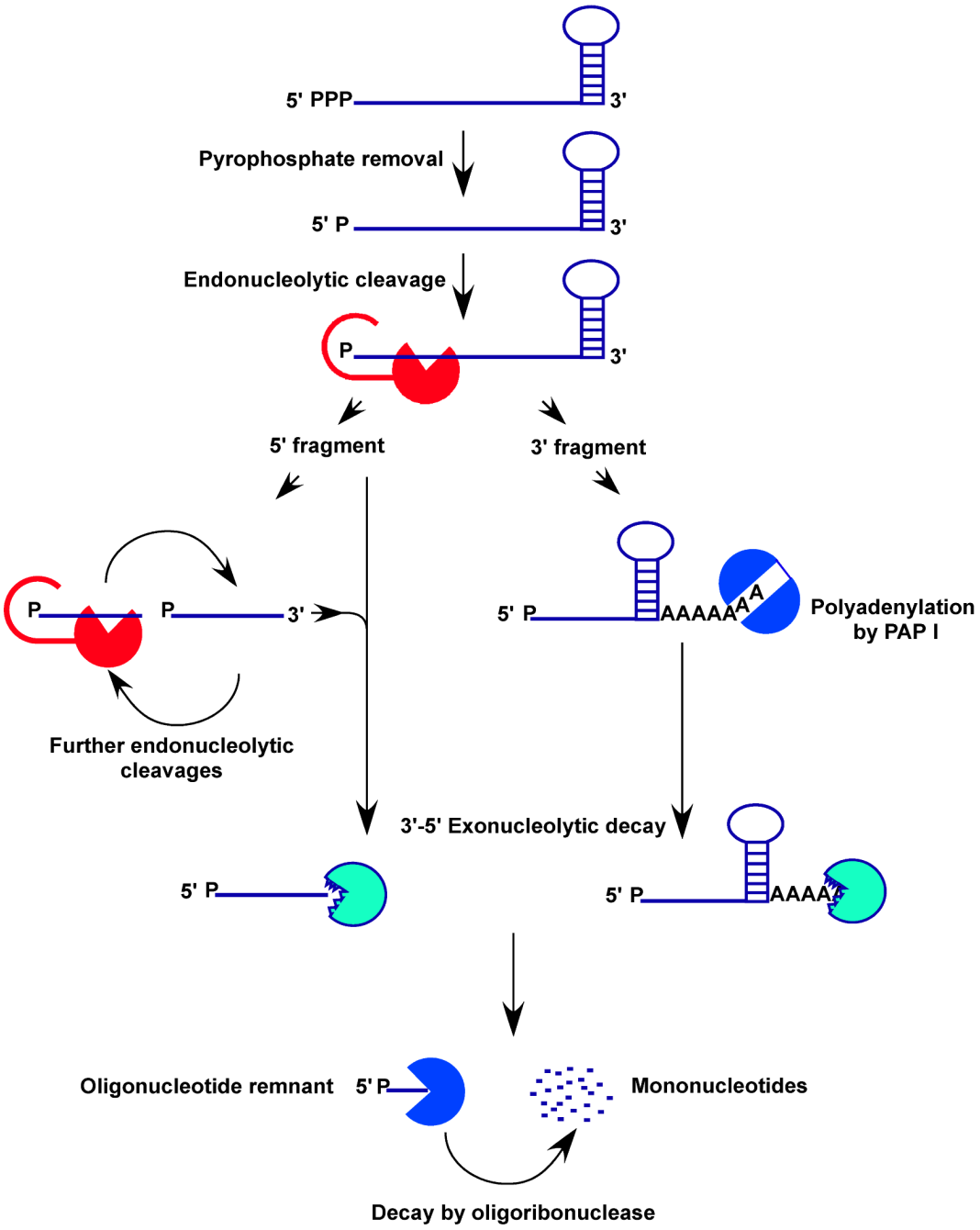

Figure 1. Schematic representation of a major pathway for mRNA decay in E. coli

A typical primary transcript possesses a single stranded, triphosphorylated 5'-terminus and a 3'-end with a stem-loop structure. Decay is initiated by the RppH-dependent pyrophosphate removal step at the 5'-terminus. Internal, endonucleolytic cleavages are performed by RNase E, which requires the monophosphorylated 5'-end for catalytic activity. The monophosphorylated 5'-fragment is then subject to further endonucleolytic cleavages or 3'-5' exonucleolytic decay by exoribonucleases such as RNase II, RNase R, and PNPase. Fragments that contain a 3' stem-loop structure are polyadenylated by poly (A) polymerase (PAP I), allowing 3'-5' exonucleolytic decay to be initiated by PNPase or RNase R. The final

oligoribonucleotide product of this process is degraded to individual nucleotides by oligoribonuclease.

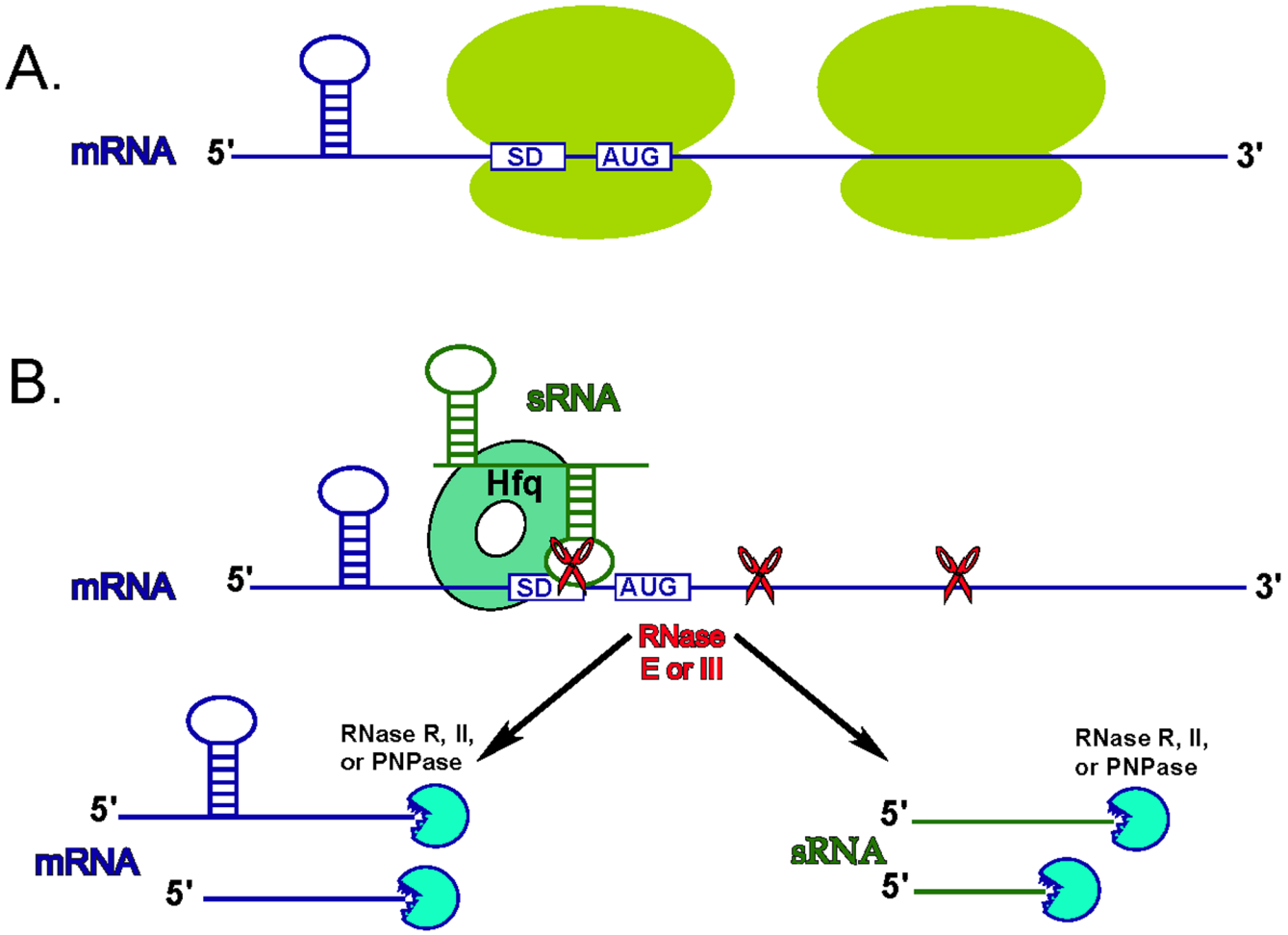

Figure 2. sRNA-mediated translation repression and mRNA decay

A. In the absence of sRNA, translating ribosomes protect mRNA from endonucleolytic degradation by blocking endonuclease sensitive sites. **B.** sRNA regulator is expressed and bound by Hfq, or another chaperone protein, that stabilizes it and facilitates its binding to the vicinity of the Shine-Delgarno (SD) element and initiation codon of the target mRNA. sRNA binding leads to cleavage of both the mRNA and sRNA by RNase III and/or RNase E. The blocking of translation might also exposes cryptic RNase E- sensitive sites within the mRNA. Initial endonucleolytic cleavage generates 5'-monophospate ends that stimulate further cleavage by RNase E, followed by 3' to 5' exonucleolytic digestion of both mRNA and sRNA remnants.

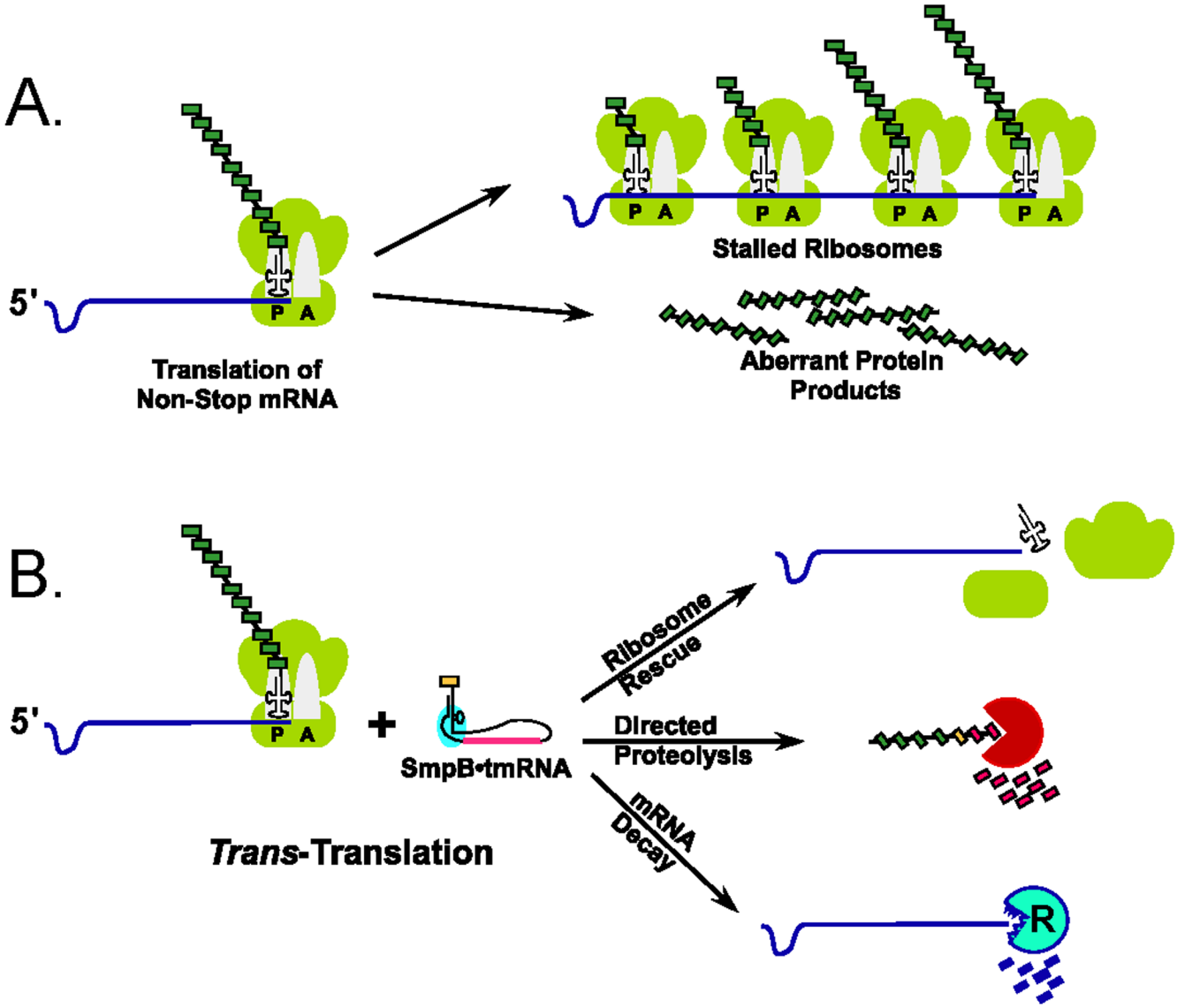

Figure 3. The consequences of translation of a non-stop mRNA, and how they are alleviated by trans-translation

A. Translation of non-stop mRNAs leads to ribosome stalling, and as mRNAs are translated by poly-ribosomes, a single stalling event can sequester many ribosomes. Furthermore, stalling could lead to the release of aberrant protein products that may be harmful to the bacterium. **B.** The SmpB•tmRNA-mediated *trans*-translation process rescues stalled ribosomes and directs the degradation of the associated incomplete protein products. The SmpB•tmRNA system also facilitates selective decay of the causative non-stop mRNA, preventing future cycles of futile translation and ribosome stalling events.