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Regulation of mRNA Decay in Bacteria

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RNase E, polynucleotide phosphorylase, RNase J, RNase Y, small regulatory RNAs, Hfq, degradosome, polyadenylation

Abstract

Gram-negative and gram-positive bacteria use a variety of enzymatic pathways to degrade mRNAs. Although several recent reviews have outlined these pathways, much less attention has been paid to the regulation of mRNA decay. The functional half-life of a particular mRNA, which affects how much protein is synthesized from it, is determined by a combination of multiple factors. These include, but are not necessarily limited to, (*a*) stability elements at either the 5′ or the 3′ terminus, (*b*) posttranscriptional modifications, (*c*) ribosome density on individual mRNAs, (*d*) small regulatory RNA (sRNA) interactions with mRNAs, (*e*) regulatory proteins that alter ribonuclease binding affinities, (*f*) the presence or absence of endonucleolytic cleavage sites, (*g*) control of intracellular ribonuclease levels, and (*h*) physical location within the cell. Changes in physiological conditions associated with environmental alterations can significantly alter the impact of these factors in the decay of a particular mRNA.

Contents

INTRODUCTION

Messenger RNA (mRNA) decay is essential for every organism because of its role in surveillance, destruction of mRNAs producing aberrant proteins, recycling of ribonucleotides, and adaptation to environmental changes. When David Apirion advanced a model for mRNA decay in *Escherichia coli* in 1973 that involved endonucleolytic cleavages and subsequent exonucleolytic degradation of the initial processing fragments (3), there was very little data to support his hypothesis. Although two 3′ → 5′ exoribonucleases [RNase II and polynucleotide phosphorylase (PNPase)] had already been discovered (59, 113) and RNase III, an endoribonuclease specific for double-stranded RNA, had been characterized (128), it was not until the identification of the essential endoribonuclease, RNase E, in the late 1970s (74, 96) that the study of mRNA decay in *E. coli* began in earnest.

During the 40 years since then, research has demonstrated that mRNA decay in both gram-negative and gram-positive bacteria predominately proceeds as hypothesized by Apirion (14, 62, 90). The recent review by Hui et al. (62) provides details on the enzymes that have been shown to be involved in mRNA decay in the two best-studied model organisms, *E. coli* and *Bacillus subtilis*.

Although every mRNA is decayed, they are not all degraded at the same rate or in the same manner. Many regulatory features play important roles to modulate mRNA decay rates. There is now sufficient information available to begin to examine potential mechanisms that are involved in the regulation of mRNA decay, although it is possible that there are additional ribonucleases and regulatory factors yet to be identified. This review focuses on the regulation of mRNA decay by a series of features: physical structure, posttranscriptional modifications, ribosome density, small regulatory RNAs (sRNAs), ribonuclease levels, intracellular location, and physiological conditions.

IMPORTANT PARTICIPANTS IN mRNA DECAY

To make it easier for the reader to follow the discussion of potential regulatory mechanisms controlling mRNA decay, we provide a brief description of some of the important players. Because of space limitations, we do not discuss minor players with overlapping functions. For more detailed information about the various enzymes, we encourage the reader to examine some of the recent excellent reviews (10, 46, 62, 135).

ENDONUCLEASES

RNase E

RNase E is an essential ribonuclease that was first identified based on its involvement in the processing of 5S rRNA (4). At about the same time, Ono & Kuwano (116) isolated a temperature-sensitive mutation that they named *ams-1* that leads to altered mRNA decay at the nonpermissive temperature. Many years later, the *rne-3071* and *ams-1* mutations were shown to be alleles of the same gene, which was subsequently named *rne* (8). After the gene was cloned (28, 37), the Rne protein was shown to contain 1,061 amino acids, with the catalytic region encompassing the first 500 amino acids (94). Additional experiments demonstrated that the carboxy terminus served as a scaffold for the association of PNPase, the RhlB RNA helicase, and the glycolytic enzyme enolase to form a multiprotein complex called the degradosome (26, 124). Each of these proteins has a specific region where it interacts with the scaffold (147). More recent work has suggested that the RNA-binding protein Hfq (see below) can also associate with RNase E, competing with the RhlB RNA helicase for the same association site (64). Crystallographic studies of the catalytic region revealed an S1 RNA–binding domain, an RNase H domain, a 5′ phosphate–binding pocket, and the catalytic domain (21). In addition, the catalytic region forms tetrameric structures in vitro (21, 67).

RNase E proteins are generally only found in gram-negative bacteria. Furthermore, in *E. coli*, where the enzyme has been studied in greatest detail, it is involved in all aspects of posttranscriptional RNA metabolism, including the maturation of tRNAs (82, 118), rRNA processing (4), and sRNA processing and decay (76). A number of experiments have suggested that the essential function of RNase E is not mRNA decay, but processing primary tRNA transcripts (118, 121). However, a recent study has indicated that the decay of specific mRNAs may be another essential function of RNase E (61).

Experiments with microarrays and high-density tiling arrays have shown that RNase E is involved in the initiation of mRNA decay in ~60% of the *E. coli* transcriptome (15, 139). Recent RNAseq experiments have mapped ~1,800 in vitro RNase E cleavage sites (36). While it was initially thought that the presence of the degradosome was required for mRNA decay, experiments with scaffold deletion mutants strongly suggest that mRNA decay for the bulk of *E. coli* transcripts proceeds normally in the absence of degradosome assembly (119). Furthermore, as noted in the next section, many organisms have orthologs of RNase E (RNase G proteins), which completely

lack a scaffold region, as the primary endoribonuclease. RNase E not only is associated with the inner membrane through an interaction of a specific eleven amino acid portion of the N-terminal protein (71), but also can rapidly diffuse along the membrane (142).

RNase G

RNase G, an ortholog of RNase E, has a limited number of mRNA substrates (77, 120) and can weakly substitute for RNase E in the processing of some tRNAs (35, 102). Initially named CafA, it was identified in a strain of *E. coli* where its overexpression led to the formation of axial filaments (115). Although the exact reason for such filament formation is unknown, it is believed to be part of cytoskeletal structure and may be involved in cell division and/or chromosomal segregation. Subsequently, several groups showed that the CafA protein encoded an endoribonuclease that had extensive homology in its catalytic region to RNase E (84, 151) but was completely lacking a scaffold region. Although its in vitro substrate specificity is similar to that of RNase E, RNase G processes the 5′ terminus of the pre-16S rRNA at a different site (84, 152).

Both RNase E and RNase G, because of the nature of the 5′-end binding pocket, prefer the presence of a 5′ phosphomonoester instead of the 5′ triphosphate that is found at the termini of primary transcripts (144). Although the presence of the 5′ triphosphate can inhibit the catalytic activity of both RNase E and RNase G, in many circumstances the enzymes bypass the 5′ terminus and initiate mRNA decay at internal sites (36).

RNase Y

RNase Y was identified in *B. subtilis* as an enzyme that could degrade highly structured RNA molecules (133). It plays a very important role in the initiation of mRNA decay in *B. subtilis* (44, 75, 80). This enzyme is not related to RNase E at the amino acid level but has similar cleavage specificity (133) and, like RNase E, is essential for cell viability. It is also associated with the inner membrane and the membrane-association region is essential for enzymatic activity (79). The protein lacks a scaffold region.

RNase J1/J2

RNase J1 and RNase J2 were identified as endonucleases in *B. subtilis* (48). Whereas RNase J2 is not essential for cell viability, RNase J1 is (44). Structural studies have demonstrated that RNase J1 can act both as an endonuclease and as a 5′ → 3′ exonuclease (43, 81).

RNase III

RNase III was discovered as an enzyme that could degrade double-stranded RNA (128). The enzyme was subsequently shown in vivo to release pre-16S and pre-23S rRNA species from primary 30S transcripts in *E. coli* (73). Although it was initially thought that the enzyme was not involved in mRNA decay, RNase III is required for the decay of some mRNAs (86, 117, 125). In fact, a recent high-density tiling array analysis revealed that the enzyme participates in the decay of approximately 12% of the *E. coli* transcriptome (139). In addition, RNase III is involved in the degradation of mRNAs targeted by *cis*-encoded sRNAs (149). RNase III is not essential in *E. coli* (7) but is required in *B. subtilis*. The essentiality of RNase III in *B. subtilis* is not related to its role in rRNA processing, but rather its role in degrading the *txpA* and *yonT* mRNAs, two toxin-encoding transcripts derived from Skin and SPβ prophages, when they are paired to the antisense RNA *ratA* (45).

EXONUCLEASES

PNPase

PNPase was first identified as an RNA polymerase based on its ability to synthesize RNA molecules in vitro (60). Subsequently, it was shown that the enzyme employs a reversible phosphorolytic mechanism that can degrade RNA molecules in the 3′ → 5′ direction in the presence of inorganic phosphate, releasing nucleoside diphosphates, or catalyze the template-independent synthesis of RNA molecules using nucleoside diphosphates as substrates (58). Because the intracellular level of inorganic phosphate is ~10 mM in *E. coli* (134), it was long assumed that the enzyme worked exclusively as an exonuclease.

However, in 2000 we found that PNPase functions both degradatively and biosynthetically in wild-type *E. coli* (98). Its degradative 3′ → 5′ exonuclease activity is inhibited by secondary structure, particularly double-stranded stems that contain more than five GC base pairs (137). The biosynthetic activity adds untemplated nucleotides based on in vivo pool sizes, generating polynucleotide tails that can be very long (98, 106). PNPase is highly conserved in bacteria and works biosynthetically to add polynucleotide tails in bacteria that do not contain a poly(A) polymerase I (PAP I) (136, 155). PNPase is an integral part of the RNase E degradosome (26) and the PAP I polyadenylation complex (106) in *E. coli*.

RNase II

RNase II, a 3′ → 5′ exonuclease, degrades RNA molecules using a hydrolytic mechanism that releases nucleotide monophosphates (113). The enzyme is very effective in degrading unstructured RNAs such as poly(A) tails (91) but is strongly inhibited by secondary structure (137). Not all bacteria have RNase II homologs.

RNase R

RNase R, a homolog of RNase II, has an altered active site and is not inhibited by secondary structures, but it requires a single-stranded region of 10–12 nucleotides to bind (30). Evidence has been presented that RNase R is involved in the degradation of rRNA and nonfunctional tRNAs (32). In bacteria such as *E. coli*, which has both enzymes, there is considerably more RNase II activity than RNase R activity (33). RNase R is required for the decay of repetitive extragenic palindrome (REP) elements (30).

In *E. coli* some mRNAs are stabilized in the absence of a particular exoribonuclease in an otherwise wild-type genetic background, suggesting that initiation of decay for such transcripts is exonuclease dependent. PNPase affects the steady-state levels of 5–17% of the expressed open reading frames (ORFs) in *E. coli*; RNase R and RNase II affect steady-state levels of ~6% and 2–7%, respectively (100, 123). Interestingly, RNase II also appears to stabilize a significant fraction (31%) of mRNAs, most likely by binding to the 3′ termini of transcripts that have Rho-independent transcription terminators (100), thus protecting them from the action of PNPase.

RNase J

Although it was generally accepted that bacteria did not contain any 5′ → 3′ exonucleases, RNase J1, originally identified as an endonuclease (48), acts as both an endonuclease and a 5′ → 3′ exonuclease that prefers 5′ monophosphorylated termini (92, 127). This class of enzyme is found in a large number of gram-positive bacteria (62) but is not found in gram-negative bacteria. It appears that in vivo RNase J1 acts primarily as a 5′ → 3′ exonuclease.

Oligoribonuclease

Biochemical analyses of RNase R, RNase II, and PNPase, have shown that none of these 3′ → 5′ exonucleases can completely degrade their RNA substrates. They leave 5′-terminal oligonucleotides ranging in length from 2 to 5 nucleotides (30, 31). Oligoribonuclease, present in many bacteria, specifically targets RNA species of ≤5 nucleotides (38, 112) to generate mononucleotides (53) and prevent them from being used to initiate new transcripts (55). The enzyme, encoded by the *orn* gene, appears to be essential in *E. coli* (53) but is not found in all bacteria. Some bacterial species contain a distinct exonuclease (NrnA/B or NrnC) that has similar properties to Orn (49, 88, 95).

OTHER PROTEINS OR ENZYMES INVOLVED IN mRNA DECAY

Poly(A) Polymerase I

In the early 1960s *E. coli* PAP I was the first poly(A) polymerase from any organism to be identified (6), but the structural gene for the enzyme (*pcnB*) was not identified until 1992 (23). It was subsequently shown that polyadenylation of mRNAs leads to their more rapid decay and that addition of poly(A) tails serves as a targeting signal for ribonucleases (97, 114). Many gram-negative bacteria have a true poly(A) polymerase, but there is no ortholog of this enzyme in *B. subtilis.*

Although transcripts for a majority of the *E. coli* ORFs are polyadenylated, only a small fraction of any specific mRNA is polyadenylated at any given time (101). Overexpression of PAP I in *E. coli* increases the level of polyadenylated transcripts significantly (97, 101), suggesting that limited polyadenylation is primarily due to very low levels of PAP I (20–50 copies/cell) (106). In contrast, excess PAP I results in polyadenylation of mature tRNAs, inhibition of protein synthesis, and cell death (105).

While any RNA molecule can be polyadenylated in vitro (156), full-length mRNAs that end with a Rho-independent transcription terminator appear to be targets for PAP I–dependent polyadenylation in vivo (101, 106). Similarly, full-length primary tRNA transcripts ending with a Rho-independent transcription terminator are primarily polyadenylated after the terminator (102, 104). A macroarray analysis of the polyadenylated *E. coli* transcriptome (101) found that multiple ORFs within polycistronic mRNAs ending with Rho-independent transcription terminators could also be targets for polyadenylation. However, a recent study showed that the polyadenylation of upstream ORFs in polycistronic transcripts can vary significantly, whereas the ORF immediately upstream of the terminator is primarily polyadenylated after the terminator (95a). Furthermore, the available data suggest that not all Rho-independent transcription terminators are targets for PAP I–dependent polyadenylation. For example, no polyadenylation was detected for the *rplY*, *rplT*, and *rpmG* transcripts (101), which contain Rho-independent transcription terminators that lack 3′ single-stranded extensions (103). In contrast, all the Rho-independent transcription terminators containing poly(A) tails had single-stranded extensions of 2–6 nucleotides (102, 103, 104).

In *E. coli*, PAP I is found in a multiprotein complex that includes the RNA-binding protein Hfq and PNPase (106). Recent data suggest that an RNase E–based degradosome regulates PAP I–dependent polyadenylation of both mono- and polycistronic mRNAs ending with a Rho-independent transcription terminator by significantly reducing the availability of free PNPase, which would compete with the PAP I polyadenylation complex (95a). Perhaps the most surprising aspect of these observations is that a functional PNPase protein is required as part of the degradosome for PAP I–dependent polyadenylation after a Rho-independent transcription terminator (95a). More than 15 years ago, *E. coli* PAP I was shown to polyadenylate many

stable RNA precursors (83); more recently it was shown to polyadenylate 79/86 pre-tRNAs in *E. coli* and to be involved in the regulation of functional tRNA levels (107).

Hfq

Hfq is a small RNA-binding protein that was first discovered based on its requirement for the replication of bacteriophage Q_β (51). Its disc-like structure has one binding face that prefers A/U-rich regions in mRNA molecules, whereas the other binding face interacts with sRNA molecules (87, 132). However, the role of Hfq in mRNA decay is most likely indirect, because the half-lives of many mRNAs are regulated under a variety of physiological conditions by small regulatory RNAs (sRNAs), and Hfq is required to facilitate the pairing of most *trans*-acting sRNAs to their target mRNAs (57).

RNA Pyrophosphohydrolase

In *E. coli* both RNase E and RNase G are inhibited by the presence of 5′ triphosphates (144), which essentially serve as caps on prokaryotic mRNAs. Furthermore, the RNase J1 5′ → 3′ exonuclease prefers 5′ monophosphorylated substrates (48). These observations led to the hypothesis that some type of decapping enzyme existed that could convert the triphosphate into a monophosphate. The Belasco group (39, 50) identified a *mutT* homolog in *E. coli* called *rppH* that encodes an RNA pyrophosphohydrolase, which can rapidly carry out this reaction on certain primary mRNA transcripts. Surprisingly, a microarray analysis showed that only a relatively small number of *E. coli* mRNAs had their steady-state levels altered in the absence of the RppH protein (39), although more recent experiments suggest that the effect of RppH may depend on the genetic background (K.E. Bowden, N.S. Weise, B.K. Mohanty, S.R. Kushner; unpublished manuscript).

Given that analysis of both the *E. coli* and the *B. subtilis* enzymes has shown that the enzymes have distinct preferences for which nucleotides are at the 5′ terminus (50, 126), only some mRNAs will be dephosphorylated. Furthermore, the requirement of conversion of 5′ triphosphate to monophosphate by RppH-like enzymes in many cases is probably avoided by a direct entry mechanism employed by ribonucleases like RNase E on a majority of its targets (36). It has now been shown that there are two RppH orthologs in *B. subtilis* (127).

RraA

The RraA (regulator of ribonuclease activity A) protein interacts with RNase E to inhibit its ability to cleave mRNAs (78). It binds to a discontinuous region of the C-terminal portion of the protein and occludes RNA binding sites, which represses the activity of the RhlB RNA helicase and, indirectly, PNPase (56). The protein can also inhibit several other DEAD box RNA helicases and appears to be a complex regulator of degradosome components (56).

RNA STRUCTURE

The presence of secondary structures at either end of an mRNA can heavily influence its stability. The coupling of transcription and translation in bacteria ensures the presence of ribosomes and RNA-binding proteins (i.e., RNA polymerase, Hfq, etc.) bound to an mRNA, which provide some measure of protection from the action of endonucleases. A high density of ribosomes on heavily translated mRNAs may effectively preclude endoribonucleolytic activity (18). However, the ribosome-free regions, such as 5′ and 3′ untranslated regions (UTRs) are always susceptible to exo- or endonucleolytic attack, making transcripts inherently unstable (34, 68). Although many

bacterial transcripts have relatively short 5′ UTRs, well-documented cases, such as the *ompA*, *rne*, and *pnp* mRNAs, have highly structured 5′ UTRs (17, 66, 131).

In the case of the *ompA* mRNA, the 5′ UTR serves as a stability element that increases the half-life by a factor of >3 (47), since its removal reduces the half-life from ~13 min to less than 4 min. Furthermore, when the *ompA* 5′ UTR is spliced onto the β-lactamase mRNA, it leads to a significant increase in its half-life (47). Experiments have shown that this stabilization requires base pairing at the 5′ terminus (5). Similar experiments in *B. subtilis* have also demonstrated that 5′ regions can lead to the stabilization of specific mRNAs (13). It seems likely that the presence of highly structured 5′ regions inhibits the binding of 5′ end–dependent ribonucleases such as RNase E; perhaps they also block their ability to act via a direct entry mechanism (9). In *B. subtilis*, however, mRNAs with a 5′ monophosphate terminus are degraded by RNase J1 even if there is secondary structure in the 5′ UTR (127).

Structures at the 3′ ends of transcripts also act as stability elements but for different reasons. Transcripts that contain Rho-independent transcription terminators have stem-loop structures with very short single-stranded regions at their 3′ termini that are insufficient for 3′ → 5′ exonuclease binding. Consequently, Rho-independent transcription terminators will inhibit the activity of RNase R, which requires a single-stranded region of 10–12 nucleotides for binding (148), and to a lesser degree for PNPase (137). Once the secondary structure is removed by an endonuclease, the resulting RNA intermediates disappear very quickly, which has been described as the all-or-none behavior of mRNA decay (138). This rapid turnover is facilitated by an RNA-degrading machine, such as RNase E–based degradosome, which contains both an endonuclease (RNase E) and a 3′→5′ exonuclease (PNPase) (138). In contrast, if an mRNA does not contain any endonuclease cleavage sites, the Rho-independent transcription terminator will provide a strong stabilizing effect in gram-negative bacteria. In fact, it appears that attempts by RNase II to initiate decay by binding at the 3′ termini of a significant number of mRNAs actually protects them from further decay (100), since the progress of RNase II is blocked by the presence of secondary structure. Furthermore, although it has been shown that PNPase can degrade certain stem-loop structures in vitro (137), it was only recently demonstrated that it could carry out this reaction in vivo in the absence of RhlB RNA helicase activity (104). On the other hand, since Rho-dependent terminators do not contain any significant secondary structures, mRNAs terminated in this fashion will be susceptible to 3′ → 5′ exonucleolytic attack by RNase II, PNPase or RNase R.

A second type of secondary structure is REP, which is observed in the spacer regions of some polycistronic messages. These are structural barriers that protect 5′ proximal transcripts from 3′ → 5′ exonucleases through the formation of stable secondary structures (110, 111). The degradation of REP sequences requires both PNPase and the DEAD-box RNA helicase RhlB, along with repeated polyadenylation by PAP I (70).

POSTTRANSCRIPTIONAL MODIFICATIONS

Many mRNA species are posttranscriptionally modified by addition of 3′ untemplated nucleotides that influence their stability. Unlike eukaryotes, which only contain poly(A) or poly(U) tails, bacteria have both poly(A) tails and polynucleotide tails (containing all nucleotides but A rich) (103). While the addition of poly(A) tails to eukaryotic mRNAs serves to stabilize the transcripts and promote their more efficient translation (154), in *E. coli* polyadenylation serves to target species for more rapid degradation (97, 114). Although only a small fraction of mRNAs are polyadenylated at any given time (estimates range between 1–2%), inactivation of poly(A) polymerase leads to significant increases in the half-lives of many mRNAs terminated with Rho-independent transcription terminators (101, 114).

Given that a very large fraction of *E. coli* transcripts are terminated in a Rho-independent fashion (52) and the activities of all three of the major 3′ → 5′ exonucleases are inhibited by either the presence of a stem (RNase II and PNPase) (137) or the absence of a long single-stranded region (RNase R) (148), it is not surprising that polyadenylation of these types of mRNAs will stimulate their decay. In the case of RNase R, the presence of a poly(A) tail permits efficient binding and the presence of secondary structure does not inhibit enzyme activity (148, 158). With PNPase, the presence of a poly(A) tail can assist the enzyme in degrading through the stem-loop associated with the terminator, although it now seems that in some cases polyadenylation may not be necessary if a longer 3′ extension is available (104).

PNPase, which is highly conserved in both gram-negative and gram-positive species, adds tails (polynucleotide) that are A rich but contain all four nucleotides. They vary in length from 20 to >600 nucleotides, and computer modeling does not predict any significant secondary structures (103). It has been suggested that the unstructured sequences provide a similar function to poly(A) tails (16, 103). Although no experimental evidence is available, this hypothesis is supported by their association with mRNA decay intermediates, mostly at sites near the 5′ ends of the transcripts (98, 106). However, it is not clear why polynucleotide tails are so much longer than poly(A) tails and whether they actually function similarly to poly(A) tails. In fact, polynucleotide tails may not be a targeting mechanism but rather a function of changes in inorganic phosphate concentration following the processive degradation of an mRNA, leading to a significant drop in localized inorganic phosphate concentration and a concomitant stimulation of the biosynthetic reaction.

Poly(A) polymerases are found in a wide variety of gram-negative bacteria, such as *Shigella*, *Salmonella*, *Yersinia*, and *Citrobacter* species, but they are not found in gram-positive bacteria. One possible explanation for their absence is the presence of RNase J1, an enzyme with 5′ → 3′ exonucleolytic activity, such that Rho-independent transcription terminators will not block mRNA decay. Given that there is a high degree of sequence conservation among the poly(A) polymerases found in gram-negative bacteria, it is not clear whether gram-positive species have lost the gene or they never had it in the first place. It should be noted that some poly(A) tails have been seen in *B. subtilis*, but their origin is unclear because there is no poly(A) polymerase ortholog in the organism and the tails are still present in a PNPase-deficient strain (22).

ALTERATIONS IN INTRACELLULAR RIBONUCLEASE LEVELS

In *E. coli* the intracellular levels of the major enzymes involved in mRNA decay are controlled in part by autoregulation [RNase E, PNPase and RNase III (12, 19, 27, 66, 93, 131)], polyadenylation [RNase E, PNPase (99)], stress conditions such as cold shock [RNase R (29)], or protein factors [RNase II (157)]. In addition, the regulatory protein RraA downregulates RNase E activity by binding to its C terminus, causing the stabilization of several RNase E–dependent transcripts (56, 78). These data suggest that control of the levels of various ribonucleases plays a significant role in the regulation of mRNA decay in *E. coli* and most likely in many other bacteria. However, under normal circumstances there appears to be excess RNase E in the cell, because enzyme levels have to be significantly reduced to see any phenotypic effect (65).

INTERACTIONS WITH SMALL REGULATORY RNAS

sRNAs are found in both gram-negative and gram-positive bacteria. These molecules fall into two categories: *cis*-acting sRNAs, which are encoded on the strand complementary to their target mRNA, and *trans*-acting sRNAs, which act at a distance and can have multiple mRNA targets (140, 153). The majority of *trans*-acting sRNAs require the presence of the RNA-binding protein Hfq

(150) to promote their ability to interact with their targets. Recent studies suggest that PNPase is responsible for degrading the sRNAs not bound to Hfq in stationary phase and protects them from RNase E degradation in exponential phase (2, 11). Bacterial sRNAs usually modulate mRNA stability by base pairing with a target mRNA at or around a ribosome binding site (RBS), inhibiting translation and stimulating their degradation, mostly by RNase E (25, 57). Recently, additional targets of various sRNAs have been identified (41).

sRNAs are generally present at very low levels in exponentially growing cells, but their levels increase dramatically under a variety of stress conditions, such as nutrient limitation, change in pH, and iron or nitrogen starvation (57). Presently, ~100 sRNAs in *E. coli* and *Salmonella typhimurium* have been documented (141). However, tiling array data derived from an RNase E deletion mutant suggests that there could be as many as 200–300 additional sRNAs (139). Many of these sRNAs, such as RyhB and MicC, have multiple targets. Thus, it would appear that a significant fraction, perhaps as high as 50%, of the mRNAs interact with at least one sRNA under the appropriate physiological conditions. Very little is known about small RNAs in gram-positive bacteria (129).

PHYSICAL LOCATION

With the development of powerful imaging technologies in the last decade, the long-held non-compartmentalized view of the bacterial cell is rapidly changing to one of highly organized, specific subcellular domains with similarities to a eukaryotic cell with organelles (1). Furthermore, demonstrations of RNA localization to particular domains within a bacterial cell have complicated the understanding of the process of posttranscriptional regulation and degradation. Four patterns of RNA localization have been identified: (*a*) in foci near transcribed genes, (*b*) throughout the cytoplasm around the cell periphery, (*c*) at the cell poles, and (*d*) in a helix-like pattern (20, 69, 108, 109) (**Figure 1**). The deduced patterns are based on studies using either *E. coli* or *Caulobacter crescentus*. The wide variations in RNA localization relative to their protein products have challenged the concept of transcription-coupled translation for some bacterial transcripts, but at the same time

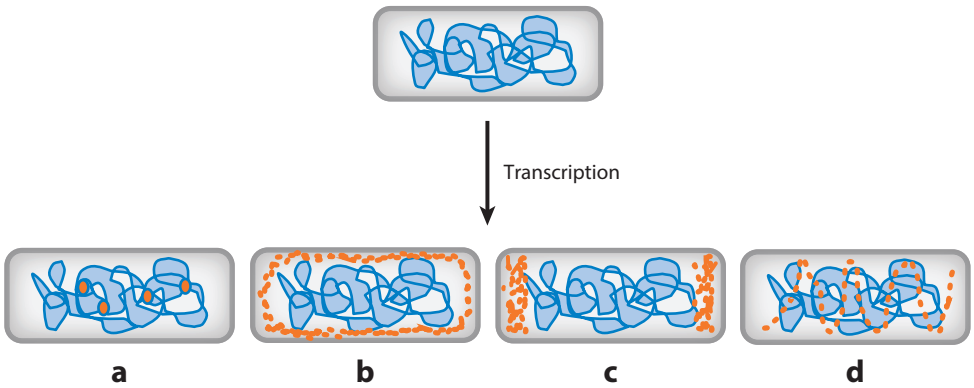

Figure 1

Graphical presentation of observed RNA localization patterns in bacterial cells. The bacterial chromosome in association with various proteins (nucleoid) are shown in blue. The RNA molecules are shown in orange. Following transcription, RNAs are localized differentially in one of four distinct patterns: (*a*) at foci near transcribed genes (54), (*b*) throughout the cytoplasm in the cell periphery (146), (*c*) at or near both cell poles (145), or (*d*) in a helix-like pattern (130).

they possibly help explain the wide variation in mRNA half-lives. It should be noted that since all of the studies were carried out using different fluorescence techniques, the conclusions regarding localization of a particular transcript were sometimes different. For example, Valencia-Burton et al. (145) showed that the *lacZ* mRNA is distributed uniformly in the cytoplasm. However, Montero Llopis et al. (108) showed that the same RNA is localized near the site of transcription, which suggests transcription-coupled translation. While this discrepancy may be attributed to use of different imaging techniques, the contrasting localization patterns suggest that the dynamic movement is not identical among all transcripts. Furthermore, the movement of mRNAs encoding membrane proteins is expected to be different from that of mRNAs encoding cytoplasmic proteins (85).

The nucleoid of a typical bacterium occupies up to 60% of the bacterium's volume. When one takes into account that in actively growing cells DNA replication and transcription are occurring, the physical volume associated with the genome is probably greater. Even with the coupling of transcription and translation, it is very likely that many mRNAs are going to be very close to the inner membrane. At least two of the four RNA localization patterns are consistent with this hypothesis (**Figure 1**). Furthermore, despite the use of different ribonucleases in *E. coli* and *B. subtilis*, many of the major enzymes involved in mRNA decay in both bacteria, such as RNase E and RNase Y, are located at the cytoplasmic membrane (63, 71, 143). In addition, RNase II, Hfq, and PAP I are present at the periphery (24, 42, 89). Given that the compaction of bacterial genomes is not random, it is likely that the physical location of a particular gene on the genome will affect the half-life of its mRNA. The mRNAs of genes located at internal locations on the nucleoid will have a lower probability of being near the inner membrane. The decay of these transcripts will be governed in part by enzymes other than RNase E and RNase Y or by how rapidly they diffuse toward the inner membrane. Thus the degradation of these transcripts may be regulated by exonucleases (RNase R or PNPase) or endonucleases such as RNase G, RNase Z, RNase III, or others that are not associated with the inner membrane.

The considerable differences in mRNA half-lives that have been measured in both *E. coli* and *B. subtilis* may be due to their physical locations, and hence their accessibility to certain ribonucleases. For example, in *E. coli* the half-lives of the *lpp*, *rpsO*, *rpsT*, and *cspE* mRNAs vary from 2.1 min to more than 10 min (97, 114, 120, 122). All four transcripts are monocistronic, are similar in length, and are terminated by Rho-independent transcription terminators, but the *lpp* mRNA (>10 min half-life) shows very little dependence on RNase E compared to the other three. Could this big difference in half-life arise from the physical location of the *lpp* gene within the genome, or is it simply due to reduced effectiveness of RNase E–independent decay pathways?

EXPONENTIAL PHASE GROWTH

For many years analysis of mRNA decay has focused on what occurs in exponentially growing cells. In this phase, nutrients are abundant and the cell is not under any type of stress. It would appear that in this environment, which probably occurs infrequently in nature, decay for most mRNAs is not governed by a specific regulatory mechanism; rather, for *E. coli* it is governed primarily by their physical location relative to the inner membrane of the cell, where the RNase E–based degradosome and the polyadenylation complex are located. The presence or absence of secondary structures at either the 5′ or 3′ terminus, as well as internal secondary structures located upstream of single-stranded A/U-rich regions, may contribute to the stability of a particular transcript. A similar situation would exist in *B. subtilis*, where RNase Y plays a similar role. These facts probably account for the typical 2- to 4-min half-life of a bacterial mRNA.

For these mRNAs—such as *lpp* and *ompA*, both of which encode membrane proteins—it is possible that longer half-lives are due to both (*a*) secondary structures such as Rho-independent

transcription terminators and/or highly structured 5′ UTRs and (*b*) differences in their physical location within the cell. For example, the half-life of the *ompA* mRNA, which has a highly structured 5′ UTR as well as a Rho-independent transcription terminator, is significantly affected if the 5′ UTR is deleted (47) or polyadenylation after the Rho-independent transcription terminator is prevented (97, 114). It would thus appear that half-lives during exponential growth are likely governed by a series of factors, including secondary structures that can impede access of either exonucleases or endonucleases, secondary structures that promote endonucleolytic cleavages (36), physical location within the cell (i.e., proximity to the inner membrane), and the density of ribosomes on each transcript.

STRESS CONDITIONS

During the typical bacterial life cycle cells encounter a variety of stress conditions, such as cold shock, nutrient starvation, nitrogen starvation, and changes in pH or iron levels. Gram-positive bacteria can deal with stress by sporulating. Gram-negative bacteria do not have this option. Depending on the particular stress, cells will respond differently. Nutrient starvation leads to activation of the stringent response, resulting in the rapid cessation of rRNA synthesis and a reduction in functional ribosome formation. Fewer ribosomes results in reduced translation and larger gaps between translating ribosomes, which will likely expose more endonucleolytic cleavage sites for RNase E.

Furthermore, nutrient starvation also triggers increased synthesis of a wide variety of sRNAs (57). Increased concentrations of sRNAs will drive sRNA/mRNA interactions in the forward direction, leading to more rapid degradation of the targeted species by either RNase E or RNase III, or possibly by 3′ → 5′ exonucleases, in *E. coli*. Whereas reduction in intracellular ribosomes affects the half-lives of many transcripts, sRNA regulation targets a more specific set of mRNAs. However, stress conditions should not alter the role of inner membrane proximity in regulating mRNA decay. It should also be noted that in some of these circumstances, such as cold shock, synthesis of RNase R is significantly increased (29). Overall, under stress conditions some regulation of mRNA decay remains unaltered (proximity to the inner membrane), while there is an overlay of sRNA-regulated and ribosome depletion–regulated mRNA decay that significantly alters the degradation of a select group of transcripts whose encoded proteins are not essential under a particular set of physiological conditions.

OVERVIEW

Based on results obtained over the last 40 years, it is clear that the decay of mRNAs is primarily initiated by endonucleases such as RNase E in *E. coli* and RNase Y in *B. subtilis*. However, the half-life of a particular mRNA is probably controlled by a series of factors that include (*a*) stability elements at either the 5′ or the 3′ terminus, (*b*) polyadenylation in gram-negative bacteria, (*c*) ribosome density on the mRNA being translated (*d*) intracellular concentrations of ribonucleases, (*e*) sRNA interactions during physiological stress, (*f*) physical location within the cell, and (*g*) the presence or absence of endonucleolytic cleavage sites.

Thus, an mRNA lacking endonucleolytic cleavage sites will be degraded exonucleolytically. In gram-negative bacteria, such transcripts may require posttranscriptional addition of either poly(A) or polynucleotide tails after the secondary structures associated with Rho-independent transcription terminators to promote binding of PNPase, RNase II, or RNase R. In gram-positive bacteria that have a 5′ → 3′ exonuclease, removal of the 5′-terminal triphosphate by an

RppH-type enzyme is a necessary prerequisite for exonucleolytic decay. It is not clear whether ribosome density plays a role in 5′ → 3′ exonucleolytic decay.

For the majority of mRNAs, whose decay is initiated by either RNase E or RNase Y, stem-loops at the 3′ terminus are not an important issue. However, ribosome density is a significant factor, given that RNase E cleavage sites are composed of an A/U-rich, single-stranded region that is downstream of a secondary structure (40, 72). Furthermore, because both RNase E and RNase Y are membrane bound, proximity to the inner membrane may be the most significant factor in determining half-lives.

It should also be noted that although RNase E and RNase Y are the primary endonucleases involved in initiating mRNA decay, experiments in *E. coli* have shown that RNase G and RNase Z, which are not membrane associated, also play a role in the decay of specific transcripts (120, 122). Overall it would appear that there is not a single regulatory mechanism that controls the decay of a particular transcript. Rather, it is a series of factors that determine the duration of what is measured as either a functional or a chemical half-life.

This review would not be complete without some words of caution. Recently, it was reported that addition of rifampicin to stop transcription initiation increased the mobility of RNase E (142). Given that almost all of the published half-life data derive from studies that employed rifampicin, these results may not accurately reflect what is occurring in unperturbed cells. In addition, much of the recent work on RNase E has been done in vitro with a protein that lacks the scaffold region. This raises questions about whether the characterized biochemical mechanisms will be comparable to what happens in vivo, where RNase E is closely associated with PNPase, an exonuclease, and the RhlB RNA helicase.

FUTURE DIRECTIONS

The development of techniques such as high-density tiling arrays and RNAseq has made it possible to compare and analyze genome-wide steady-state mRNA levels under diverse physiological conditions. However, comparison of genome-wide half-lives of individual transcripts using these techniques is still a challenge. More experimental work is needed to establish which mRNAs are decayed exclusively exonucleolytically rather than by initial endonucleolytic cleavages. As more sophisticated technologies are developed it may also become possible to determine how important proximity to the inner membrane is in determining the half-life of an individual mRNA. It should also be possible to determine what role the so-called minor endonucleases—such as RNase G, RNase Z, and RNase LS—play in controlling mRNA half-lives. Finally, it will not be surprising if additional enzymes and/or regulatory factors affecting mRNA half-lives are identified in the very near future.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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