Lost in translation: the influence of ribosomes on bacterial mRNA decay

Atilio Deana and Joel G. Belasco

Skirball Institute of Biomolecular Medicine and Department of Microbiology, New York University School of Medicine, New York, New York 10016, USA

The lifetimes of bacterial mRNAs are strongly affected by their association with ribosomes. Events occurring at any stage during translation, including ribosome binding, polypeptide elongation, or translation termination, can influence the susceptibility of mRNA to ribonuclease attack. Ribosomes usually act as protective barriers that impede mRNA cleavage, but in some instances they can instead trigger the decay of the mRNA to which they are bound or send a signal that leads to widespread mRNA destabilization within a cell. The influence of translation on mRNA decay provides a quality-control mechanism for minimizing the use of poorly or improperly translated mRNAs as templates for the production of abnormal proteins that might be toxic to bacteria.

Messenger RNA degradation plays a key role in controlling gene expression in all organisms. This is particularly true in bacteria, where mRNA decay rates can differ by as much as two orders of magnitude, with half-lives ranging from a fraction of a minute to more than an hour. The lifetimes of individual mRNAs are influenced by several factors, among the most important being their association with translating ribosomes. Recent discoveries have led to a better appreciation of the variety of ways in which ribosomes can impede or accelerate bacterial mRNA degradation and the regulatory significance of their ability to do so.

Typical mechanism of mRNA decay in bacteria

Bacterial RNA transcripts initially bear a triphosphate at the 5’ end and a stem-loop structure at the 3’ end. In Escherichia coli, the degradation of most mRNAs is thought to begin with internal cleavage by RNase E, an endonuclease that cuts RNA in single-stranded regions. In E. coli, the degradation of most mRNAs is thought to begin with internal cleavage by RNase E, an endonuclease that cuts RNA in single-stranded regions that are AU-rich (Mudd et al. 1990; Babitzke and Kushner 1991; Melefors and von Gabain 1991; Taraseviciene et al. 1991; McDowall et al. 1994). Less frequently, mRNA decay in E. coli starts with cleavage by another endonuclease, such as RNase III, RNase G, or RNase P (Schmeissner et al. 1984; Portier et al. 1987; Umitsuki et al. 2001; Li and Altman 2003). No longer protected by a 3’-stem-loop, the resulting upstream cleavage products are promptly degraded by 3’-exonucleases, which can proceed all the way to the 5’ end unless they encounter an intercistronic stem-loop of significant thermodynamic stability. The downstream products of endonuclease cleavage also undergo rapid endonucleolytic and 3’-exonucleolytic degradation owing to their monophosphorylated 5’ termini, which render them highly susceptible to further digestion due to the preference of RNase E and poly(A) polymerase (a facilitator of 3’-exonuclease digestion) for monophosphorylated RNA substrates [Mackie 1998; Feng and Cohen 2000]. 5’-exonucleases are not thought to participate in bacterial mRNA degradation, as no such ribonuclease has been identified in any prokaryotic organism.

The disparate cytoplasmic lifetimes of bacterial mRNAs appear to be strongly affected by characteristics of the 5’-untranslated region (UTR), as evidenced by the stabilizing influence of 5’-UTRs on long-lived mRNAs (e.g., E. coli ompA, which encodes an outer membrane protein, or Bacillus subtilis aprE, which encodes subtilisin) on more labile mRNAs to which they are fused [Belasco et al. 1986; Hambraeus et al. 2000]. The key features of these 5’-UTRs that enable them to protect mRNA from degradation include their ribosome-binding site (RBS, see below) and the presence of a stem-loop structure at the 5’ terminus [Emory et al. 1992; Arnold et al. 1998; Hambraeus et al. 2002]. These findings have led to the conclusion that there is an important pathway for bacterial mRNA degradation in which bound ribosomes or base-pairing near the 5’ end can impede access to internal endonuclease cleavage sites by a mechanism that is poorly understood. In other cases, the degradation machinery may bypass the 5’ end and access internal cleavage sites directly [Fig. 1, Joyce and Dreyfus 1998, Baker and Mackie 2003].

Influence of ribosome binding and translation initiation on mRNA decay

Some of the earliest evidence as to the effect of bound ribosomes on mRNA decay came from studies with an-
tibiotic inhibitors of translation. However, the results of those experiments were later judged to be difficult to interpret due to indirect, cell-wide effects of these drugs unrelated to their impact on translation of the mRNAs being investigated [Lopez et al. 1998].

Ribosome-binding site occupancy

More reliable information has come from examining the influence of RBS mutations on RNA decay. For example, experiments in both E. coli and B. subtilis have shown that a variety of mRNAs can be significantly destabilized by mutations in the Shine-Dalgarno element that interfere with ribosome binding by markedly reducing its complementarity to 16S rRNA [Wagner et al. 1994; Arnold et al. 1998; Jürgen et al. 1998; Hambraeus et al. 2002; Sharp and Bechhofer 2003]. In E. coli, the accelerated decay of such translationally impaired mRNAs is generally mediated by RNase E [Jain and Kleckner 1993; Arnold et al. 1998]. Conversely, mutations that improve ribosome binding can prolong mRNA longevity [Jain and Kleckner 1993; Matsunaga et al. 1997]. Together, these findings indicate that ribosome binding to the RBS helps to protect mRNAs from ribonuclease attack. The importance of ribosome binding for mRNA longevity probably contributes to the lability of mRNA decay intermediates that lack an RBS, a property that helps to prevent the cytoplasmic accumulation of RNA fragments.

Possible explanations for the destabilizing effect of mutations that impair ribosome binding are of two kinds [Arnold et al. 1998; Baker and Mackie 2003; Dreyfus and Joyce 2003]. According to one interpretation, by directly stimulating translation initiation, a better RBS would allow closer spacing of translating ribosomes and thereby improve steric protection of potential RNase E cleavage sites in or near the protein-coding region (Fig. 1, top). An alternative interpretation is based on evidence that mRNA features near the 5′ terminus can control access to internal RNase E cleavage sites by a mechanism that is not yet understood. The presence of a ribosome at the RBS or a stem-loop at the 5′ end can impede such access and protect the mRNA from attack. For many transcripts, these two pathways may be able to operate concurrently, albeit at different rates; therefore, either the absence of a 5′-terminal barrier or the unmasking of internal sites could facilitate cleavage of such mRNAs. [Scissors] RNase E; [cylinder] protein-coding region; [X] potential RNase E cleavage site. Arrows identify accessible cleavage sites.

Effects of translation on mRNA decay
significantly diminishing the ribosome-binding affinity of the RBS and its average occupancy by ribosomes. In *E. coli* and *B. subtilis*, changing an initiation codon from AUG to a weaker initiation codon (GUG, CUG, or UUG) or to a codon that cannot function in translation initiation typically results in a marked reduction in translational efficiency, yet more often than not, such changes cause only a modest decrease in mRNA half-life (Wagner et al. 1994; Matsunaga et al. 1997; Arnold et al. 1998; Hambraeus et al. 2002, Sharp and Bechhofer 2003; Komarova et al. 2005). These findings suggest that a high degree of RBS occupancy by ribosomes is usually more important for mRNA longevity than is close spacing of translating ribosomes.

**Repression of translation by protein binding**

If a poor Shine-Dalgarno element can destabilize mRNA by impairing ribosome binding, then other impediments to ribosome binding might be expected to have a similar effect. For example, the binding of ribosomes to even a good RBS can be inhibited by competitive binding of a regulatory protein. Such inhibition is typically accompanied by a reduction in mRNA longevity. Thus, the Sm-like protein Hfq and ribosomal protein L1 (the rplA gene product) each autoregulate their expression by binding their own mRNA in the vicinity of the RBS (the hfg RBS or the 5‘-proximal rplK RBS of the polycistronic rplK/AJL transcript). The resulting decrease in ribosome binding causes a marked increase in the decay rate of these mRNAs (Cole and Nomura 1986; Včèrek et al. 2005). Feedback regulation of the *E. coli* *thtS* (threonyl tRNA synthetase) gene has also been reported to involve coordinated effects of ThtS protein binding on translation initiation and mRNA stability (Nogueira et al. 2001).

**Repression of translation by base-pairing**

Ribosome binding can also be competitively inhibited via occlusion of the RBS by intra- or intermolecular base-pairing, with deleterious consequences for mRNA longevity. For example, mutations outside the RBS that increase the potential for base-pairing of the RBS with the mutated RNA segment have been found to accelerate RNase E-mediated mRNA degradation by interfering with ribosome binding, whereas mutations that weaken RBS base-pairing have the opposite effect (Cho and Yanofsky 1988, Yarchuk et al. 1992). Members of a recently discovered class of cis-acting RNA regulatory elements are likely to have a similar influence on mRNA stability. These “riboswitches” each bind selectively to a small cellular metabolite, such as a vitamin or amino acid that induces a conformational change in the surrounding RNA structure. In a number of cases (e.g., the *E. coli* *bthB* and *thitB* mRNAs and the *B. subtilis* *ypaA* mRNA), this structural reorganization inhibits translation initiation by sequestering the RBS (Nou and Kadner 2000, Winkler et al. 2002a,b). Although not yet reported, there is every reason to expect the lifetimes of these mRNAs to be significantly diminished in the translationally repressed conformation.

Repression of translation initiation by intermolecular base-pairing can also accelerate mRNA decay. Several small untranslated RNAs (sRNAs) have been shown to control gene expression by base-pairing at or near the RBS of target mRNAs (Gottesman 2004). Many of these sRNAs mediate the regulatory influence of environmental signals such as cell density, iron starvation, temperature, oxidative stress, or phosphosugar stress, and they do so very effectively despite being imperfectly complementary to the mRNA elements with which they anneal. sRNAs are thought, in many cases, to be delivered to their mRNA targets by Hfq, whose presence can also help to protect these sRNAs from degradation (Sledjeski et al. 2001, Massé et al. 2003; Moll et al. 2003; Geissmann and Touati 2004). Two well-characterized paradigms for this important class of regulatory RNAs are RyhB, a 90-nucleotide (nt) sRNA produced when iron is scarce, and DsrA, an 87-nt sRNA produced at low temperatures. In *E. coli*, RyhB represses the synthesis of several proteins that store or use iron, including superoxide dismutase (the sodB gene product). Masking of the *sodB* RBS by intermolecular base-pairing with RyhB downregulates *sodB* translation and accelerates degradation of this mRNA by RNase E (Massé et al. 2003). Conversely, DsrA sRNA unmasks the RBS of *rpoS* mRNA (which encodes a stationary-phase/stress-response σ factor) by annealing to an upstream region that otherwise occludes the RBS by intramolecular base-pairing. As a consequence of interacting with DsrA, translation of *rpoS* mRNA is enhanced and its lifetime is prolonged (Lease and Belfort 2000). Other sRNAs are likely to influence mRNA translation and decay via similar mechanisms.

A number of other mRNAs (e.g., the IS10 transposase and plasmid R1 repA transcripts) are perfectly complementary to the sRNAs with which they interact because both are transcribed from opposite strands of the same genetic locus. Annealing of the antisense RNA to the RBS of these mRNAs not only inhibits ribosome binding but also creates a target for mRNA cleavage by RNase III, an endonuclease that cuts long, perfectly paired RNA duplexes and certain imperfect duplexes (Blomberg et al. 1990). Two well-characterized paralogous sequences for this important class of regulatory RNAs are DsrA and DsrB, which anneal to ribosome-binding sites on their mRNA targets by Hfq, whose presence can also help to protect these sRNAs from degradation (Sledjeski et al. 2001, Massé et al. 2003; Moll et al. 2003; Geissmann and Touati 2004). Two well-characterized paradigms for this important class of regulatory RNAs are RyhB, a 90-nucleotide (nt) sRNA produced when iron is scarce, and DsrA, an 87-nt sRNA produced at low temperatures. In *E. coli*, RyhB represses the synthesis of several proteins that store or use iron, including superoxide dismutase (the sodB gene product). Masking of the *sodB* RBS by intermolecular base-pairing with RyhB downregulates *sodB* translation and accelerates degradation of this mRNA by RNase E (Massé et al. 2003). Conversely, DsrA sRNA unmasks the RBS of *rpoS* mRNA (which encodes a stationary-phase/stress-response σ factor) by annealing to an upstream region that otherwise occludes the RBS by intramolecular base-pairing. As a consequence of interacting with DsrA, translation of *rpoS* mRNA is enhanced and its lifetime is prolonged (Lease and Belfort 2000). Other sRNAs are likely to influence mRNA translation and decay via similar mechanisms.

**Influence of translation elongation and termination on mRNA decay**

Even after they initiate protein synthesis, there are a variety of ways in which ribosomes can prolong or shorten mRNA lifetimes in response to events that occur during translation elongation or termination. These processes can affect the longevity of the specific subset of transcripts on which the events take place or generate
a signal that triggers the accelerated degradation of most mRNAs within the cell.

Premature translation termination

Nonsense mutations that cause premature translation termination frequently hasten mRNA decay, especially when the premature termination codon (PTC) is close to the RBS [Nilsson et al. 1987]. In E. coli, such destabilization has generally been attributed to accelerated RNase E cleavage in or near the coding region at sites (usually undefined) that normally are sterically protected by the periodic transit of translating ribosomes but are exposed when translation terminates at an upstream PTC [Arnold et al. 1998; Braun et al. 1998]. Consistent with this interpretation is the finding that the rate of RNase E cleavage at a site in rpsO [ribosomal protein S15] mRNA that is just downstream of the coding region depends on the distance between this site and the termination codon [Braun et al. 1998]. That a PTC can also reduce the lifetime of mRNA bearing an inefficient initiation codon suggests that even infrequent traversal of the coding region by ribosomes can help to impede degradation, perhaps by dislodging RNase E from the message before cleavage can occur [Arnold et al. 1998].

Other data suggest that the destabilizing effect of PTCs cannot always be explained so simply. For example, in E. coli the marked destabilization of bla [β-lactamase] mRNA caused by introducing a PTC near the 5′ end of the coding region is virtually abolished when this mutant mRNA is fused downstream of another translational unit to create a synthetic dicistronic transcript [Hansen et al. 1994]. This finding suggests that the proximity of the PTC to the 5′-most initiation codon may in some cases be more important for rapid decay than the unmasking of potential cleavage sites downstream of the PTC. Furthermore, some transcripts [e.g., B. subtilis aprE, Rhodobacter capsulatus psuBA] are not destabilized by PTCs, presumably because other characteristics of these mRNAs or their host organisms make them resistant to ribonuclease attack under these circumstances [Klug and Cohen 1991; Hambraeus et al. 2002, Sharp and Bechhofer 2003].

Uncoupling transcription and translation

Another way that sites within the coding region can be transiently exposed to RNase E cleavage is by uncoupling transcription and translation. Normally, translating ribosomes traverse mRNA at a rate very similar to that at which it is synthesized by E. coli RNA polymerase [20–60 nt per second] [Young and Bremer 1976; Epshtein and Nudler 2003]. As a result, nascent transcripts are thought to carry a full complement of ribosomes that follow closely behind the polymerase. In contrast, ribosomes cannot keep pace with T7 RNA polymerase, which synthesizes mRNA severalfold faster than the E. coli enzyme. Consequently, nascent transcripts produced by this bacteriophage enzyme in E. coli each contain a long ribosome-free segment between the lead ribosome and the transcribing polymerase, making them more susceptible to RNase E digestion by unmasking cleavage sites within the exposed segment [lost and Dreyfus 1995]. Whether this deficit of ribosomes also helps to accelerate RNase E cleavage by momentarily leaving the RBS unoccupied just after transcription begins and thereby deprotecting the 5′ end remains to be determined. As expected, mRNAs transcribed by T7 RNA polymerase acquire a normal lifetime once they are fully synthesized and the lead ribosome reaches the end of the coding region.

Interestingly, although the ribonuclease activity of RNase E is entirely contained within the N-terminal half of the protein [McDowell and Cohen 1996], the marked instability of nascent T7 polymerase transcripts also requires the noncatalytic carboxyl portion of RNase E [Lopez et al. 1999]. This C-terminal region contains an arginine-rich RNA-binding domain and docking sites for at least three other proteins, including a 3′ exoribonuclease, an RNA helicase, and a glycolytic enzyme [Tara and Cohen 1995; McDowell and Cohen 1996; Vanzo et al. 1998]. By analogy, it seems possible that the C-terminal half of RNase E will also be found to contribute to the accelerated decay of other mRNAs that contain long segments with little or no ribosome protection, such as those that have a premature termination codon or a weak Shine-Dalgarno element.

Stabilization by stalled ribosomes

At high concentrations, antibiotics that inhibit translation elongation [e.g., chloramphenicol, tetracycline, fusidic acid] have been found to stabilize most mRNAs in bacteria. Initially, this observation was interpreted as evidence that stalled ribosomes can inhibit mRNA degradation by sterically masking mRNA cleavage sites in close proximity and/or by blocking the 5′ end. However, the discovery that these drugs can also prolong the lifetimes of untranslated RNAs in E. coli has raised the possibility that their stabilizing effect on mRNA may, in whole or in part, be an indirect consequence of inhibiting cellular protein synthesis [Lopez et al. 1998].

At lower concentrations insufficient to arrest protein synthesis or cause a general change in mRNA longevity, erythromycin can selectively stabilize two Staphylococcus aureus mRNAs [ermA and ermC] encoding related rRNA methyltransferases that mediate resistance to this antibiotic. In each case, a low dose of erythromycin causes ribosome stalling in a short open reading frame within the 5′-leader region of the mRNA. In both S. aureus and B. subtilis, the stalled ribosome induces methyltransferase synthesis by disrupting base-pairing that would otherwise occlude the RBS; in addition, it prolongs the lifetime of ermA and ermC mRNA up to 20-fold by a mechanism independent of the increase in translation [Bechhofer and Dubnau 1987; Sandler and Weisblum 1988]. Interestingly, when the 5′ end of either erm transcript is extended by fusing additional RNA there, erythromycin-induced ribosome stalling protects
only the mRNA segment downstream of the stall site, consistent with a directional stabilization mechanism involving 5′-end blockade when a ribosome is stalled nearby (Bechhofer and Zen 1989, Sandler and Weisblum 1989). A similar mechanism has been invoked to explain how the <i>S. aureus</i> <i>cat</i> [chloramphenicol acetyltransferase] transcript is stabilized by subinhibitory concentrations of chloramphenicol (Dreher and Matzura 1991). It remains to be determined whether mRNA stabilization by ribosomes caused by antibiotics to stall during translation elongation is a phenomenon unique to Gram-positive organisms, such as <i>S. aureus</i> and <i>B. subtilis</i>, or common to all bacteria.

### Destabilization by translational pausing

It has recently been discovered that translational pausing can sometimes induce endonucleolytic mRNA cleavage near the paused ribosome. Such cleavage has been observed in certain <i>E. coli</i> transcripts at sites preceded by an RNA (or nascent polypeptide) element that delays translation elongation or termination (Loomis et al. 2001; Hayes and Sauer 2003; Sunohara et al. 2004a, b). Generally, these elements end with a proline codon (or proline residue), which is followed by a stop codon in cases of delayed translation termination, but internal clusters of rare codons decoded by low-abundance aminoacyl-tRNAs (<i>e.g.,</i> AGG codons) may have a similar effect (Sunohara et al. 2004a). Unlike mRNA cleavage triggered by ribosome pausing during translation elongation, that brought about by delayed termination appears to occur specifically within the codon at the A-site of the paused ribosome. As a result, the ribosome that induces cleavage and any ribosomes trailing it are deprived of an opportunity to terminate translation normally. Ribosome release is instead achieved by <i>trans</i>-translation with tmRNA, which directs translation termination after having tagged the C terminus of the resulting protein with an oligopeptide that mediates its swift destruction (Withney and Friedman 2003). No longer protected at its 3′ end by a trapped ribosome, the 5′ mRNA cleavage product is expected to be rapidly degraded by 3′ exonucleases.

The endonuclease activity responsible for cleaving mRNA near paused ribosomes has not been identified. Experiments with ribonuclease mutants have ruled out the usual suspects (RNase E, RNase III, and RNase G) as well as several TA (toxin-antitoxin) ribonucleases (see below), raising the possibility that a previously undiscovered endonuclease or intrinsic activity of the small ribosomal subunit may be involved (Hayes and Sauer 2003; Sunohara et al. 2004a, b). In one case, an RNA helicase (HrpA) has been shown to assist cleavage near a site where ribosomes pause during elongation (Koo et al. 2004). Interestingly, though seemingly precluded from binding there until after mRNA cleavage occurs, tmRNA can facilitate [but is not required for] mRNA cleavage in the A-site of ribosomes paused at a termination codon (Sunohara et al. 2004b).

### Widespread destabilization induced by amino acid starvation

Paused ribosomes not only can trigger the cleavage of mRNAs to which they are bound but also can function indirectly to induce accelerated decay of other cellular mRNAs by activating the degradative component of bacterial toxin–antitoxin systems. The <i>E. coli</i> chromosome encodes five such TA systems—RelE–RelB, MazF–MazE, ChpBK–ChpBI, YoeB–YefM, and YafQ–DinJ—in which the toxin is a protein capable of causing widespread mRNA degradation unless bound and inhibited by its antitoxin partner (Gerdes et al. 2005). Other bacterial genomes contain as many as 45 TA loci. Normally such toxins are maintained in an inactive state by association with their respective antitoxins, but they can be activated in cells starved for amino acids [<i>or under other stress conditions</i>], apparently as a result of decreased synthesis or increased degradation of the antitoxins (Ai-zenman et al. 1996; Christensen et al. 2001, 2003; Hazan et al. 2004). The effect on antitoxin production or turn-over presumably is a direct or indirect consequence of ribosome pausing caused by a shortage of charged tRNA. Toxin activation under conditions of amino acid starvation provides a degradative mechanism for bringing about a general reduction in protein synthesis when amioacyl-tRNAs are scarce.

Of the five <i>E. coli</i> toxins, three (MazF, ChpBK, and YoeB) have thus far been shown to possess intrinsic endonuclease activity that enables them, when purified, to cut RNA in the absence of ribosomes (Kamada and Hanaoka 2005; Zhang et al. 2005a, b). In bacterial cells, however, these toxins, as well as RelE, have been found to mediate cleavage predominantly in translated regions of mRNA, suggesting a role for ribosomes in helping to specify cutting sites (Christensen et al. 2003, 2004). This is clearly so for RelE-dependent mRNA cleavage, which occurs at codons positioned in the A-site of bound ribosomes (Pedersen et al. 2003). Thus, it appears that translation can influence both the activation and function of toxins encoded by TA loci.

The nascent polypeptide and mRNA localization

In addition to its direct effect on mRNA cleavage, translation can influence mRNA longevity indirectly by determining the cellular localization of transcripts. A recently discovered example of an mRNA whose localization affects its stability is the <i>E. coli</i> <i>ptsG</i> transcript, which encodes a transmembrane glucose transport protein [IICβ]<sub>Glc</sub>. The lifetime of <i>ptsG</i> mRNA is regulated by SgrS, a small untranslated RNA whose synthesis is induced by phosphosugar stress. By annealing to the <i>ptsG</i> RBS, SgrS inhibits translation initiation and accelerates <i>ptsG</i> mRNA degradation by RNase E (Kimata et al. 2001; Vanderpool and Gottesman 2004). Interestingly, the efficacy with which SgrS hastens the decay of <i>ptsG</i> mRNA appears to depend on the ability of translating ribosomes to bring this transcript to the cell membrane so that its protein product can be inserted cotranslation-
ally into the lipid bilayer (Kawamoto et al. 2005). This conclusion is based on the finding that the first two transmembrane helices of either IICBG1c or another integral membrane protein must be synthesized as part of the nascent polypeptide in order for SgrS to destabilize ptsG mRNA.

The mechanism by which proximity to the cell membrane facilitates SgrS-mediated degradation of the ptsG transcript has not been determined. In principle, this property could be explained if SgrS and/or a general degradation factor is unevenly distributed within the cytoplasm or if ribosomes compete less efficiently with SgrS for binding the RBS of polysomal transcripts that are membrane-associated. In this regard, it is noteworthy that the concentration of RNase E in E. coli appears to be highest near the membrane (Liou et al. 2001).

Biological importance of linking translation and mRNA degradation

The finding that impaired translation often accelerates bacterial mRNA decay raises important questions as to the selective evolutionary advantage conferred by rapidly degrading transcripts that are already functioning poorly, if at all, as templates for protein synthesis. One likely explanation is that this may serve as a quality-control mechanism to minimize the production of aberrant proteins that might be toxic to bacterial cells. Such aberrant polypeptides could be generated from individual mRNAs either by translation termination at a premature stop codon or by adventitious translation initiation at cryptic sites within the protein-coding region; in addition, they could be produced more pervasively by decreased translational fidelity when amino acids are scarce. The potential for protein synthesis from cryptic initiation sites may be significant for poorly translated mRNAs. Such sites are likely to be widespread in the coding regions of bacterial transcripts due to flexible criteria for the sequence and location of prokaryotic translation initiation sites, but they should normally be functionally repressed by frequent translational readthrough from the principal RBS (Platt et al. 1972; Schottel et al. 1984). However, if such readthrough is infrequent because the principal RBS is inefficient or because ribosomes pause or dissociate somewhere upstream, translation from these cryptic initiation sites could become significant were the transcript not rapidly degraded.

Another potential benefit is that, whereas inhibition of translation initiation by base-pairing or protein binding is a reversible repression mechanism, RNA degradation is an irreversible process that can rid cells completely of transcripts that are no longer needed and that might otherwise impose a regulatory or energy burden. Interestingly, sRNA-mediated translational repression often triggers accelerated degradation not only of the mRNA but also of the annealed sRNA by RNase E or RNase III (Case et al. 1990; Massé et al. 2003). By coupling decay of the sRNA to the completion of its regulatory mission, cells can quickly resume translation of newly synthesized target transcripts once sRNA synthesis ceases.

The relationship between translation and mRNA decay is often reciprocal, with partial degradation altering translation in ways that facilitate the further digestion of decay intermediates. Notably, mRNA cleavage can influence translation initiation by severing or structurally reorganizing an RBS, thereby hastening additional cleavage. Furthermore, because cleavage within the coding region of a transcript can prevent translating ribosomes from encountering a termination codon, bacteria have evolved a mechanism (tmRNA-mediated trans-translation) for releasing ribosomes trapped at the 3’ ends of mRNA fragments that lack a stop codon. Once freed from trapped ribosomes, the 3’ termini of these decay intermediates can be accessed by 3’ exonucleases and the intermediates degraded.

Outlook

Whatever may have been the original evolutionary imperatives for linking mRNA decay and translation, it is clear that bacteria now take advantage of the connection between these two processes as a general means for controlling levels of gene expression and for adjusting those levels in response to growth conditions and environmental signals. However, the molecular mechanisms by which ribosomes influence mRNA cleavage rates are still poorly understood. Recent progress in elucidating the structure and function of bacterial ribosomes and ribonucleases makes it reasonable to expect that deeper insights into these mechanisms may soon be at hand.

Acknowledgments

We are very grateful to Marc Dreyfus for his helpful comments. The concepts presented here are based on research from many laboratories, not all of which has been mentioned because of space constraints. The writing of this review article was supported by a research grant from the National Institutes of Health (GM35769).

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Lost in translation: the influence of ribosomes on bacterial mRNA decay

Atilio Deana and Joel G. Belasco

Genes Dev. 2005, 19:
Access the most recent version at doi:10.1101/gad.1348805

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