Translation initiation and the fate of bacterial mRNAs

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Abstract

Studies in pro- and eukaryotes have revealed that translation can determine the stability of a given messenger RNA. In bacteria, intrinsic mRNA signals can confer efficient ribosome binding, whereas translational feedback inhibition or environmental cues can interfere with this process. Such regulatory mechanisms are often controlled by RNA-binding proteins, small noncoding RNAs and structural rearrangements within the 5’ untranslated region. Here, we review molecular events occurring in the 5’ untranslated region of primarily Escherichia coli mRNAs with regard to their effects on mRNA stability.

Introduction

RNA degradation counterbalances transcription, and therefore plays an important regulatory role in adjusting the steady-state level of a given mRNA. Unlike stable ribosomal and transfer RNAs, many mRNAs are labile and their decay is frequently initiated shortly after or, in peculiar cases, even before their transcription is completed (Cannistraro & Kennell, 1985). In Escherichia coli, and presumably in many other Gram-negative bacteria, the rate of mRNA decay is dependent on initial cleavage(s) mainly performed by endoribonuclease RNase E (Melefors et al., 1993; Kushner, 2002), sometimes by RNase III (Conrad & Rauhut, 2002) or other endoribonucleases (Arraiano et al., 1993; Alifano et al., 1994; Arraiano et al., 1997; Umitsuki et al., 2001; Li & Altman, 2003). RNase E cleaves RNAs with a 5’ monophosphate group preferentially over those endowed with a 5’ triphosphate (Lin-Chao & Cohen, 1991; Mackie, 1998, 2000). Recent structural data (Callaghan et al., 2005) confirm the previous finding (Jiang et al., 2000) that the structural module discriminating mono- and triphosphate groups is located within the catalytic domain of RNase E. Because primary transcripts possess triphosphate groups, whereas the downstream products of RNase E cleavage are monophosphorylated (Misra & Apirion, 1979), the ‘5’-end dependency’ of RNase E appears to pave the way for efficient cleavage at any RNase E recognition site downstream of the initial cleavage (Mackie, 1998, 2000). Although some Gram-positive bacteria lack RNase E sequence homologues (Condon & Putzer, 2002), they apparently have functional counterparts. Putzer and coworkers have recently identified the Bacillus subtilis RNase J1/RNase J2 endoribonucleases (Even et al., 2005) that biochemically resemble E. coli RNase E.

In E. coli, RNase E provides a scaffold for a multienzyme complex termed the ‘degradosome’ that, in addition to RNase E, contains polynucleotide phosphorylase (PNPase), a 3’ → 5’ exoribonuclease, the RhlB RNA helicase, the glycolytic enzyme enolase, and a number of minor components (Carpousis, 2002). Several lines of evidence suggest the physical and functional cooperation of degradosomal proteins in the degradation and processing of E. coli RNA in vivo (Xu & Cohen, 1995; Lopez et al., 1999; Liou et al., 2001; Leroy et al., 2002; Bernstein et al., 2004; Khemici & Carpousis, 2004; Khemici et al., 2005). It is commonly accepted that the ‘5’-end dependency’ of RNase E together with the concerted action of the key ribonucleases and auxiliary enzymes such as RhlB and poly(A) polymerase I lead to efficient decay of E. coli transcripts after their initial cleavage by RNase E. As many details of the mRNA decay machinery are not covered here, the reader is referred to previous reviews on this topic (Carpousis et al., 1999;
Ribosome binding and mRNA stability

The common translation initiation pathway in bacteria, which leads to ternary complex formation between mRNA, fMet-tRNA\textsuperscript{Met} and the 30S ribosomal subunit, is kinetically controlled by three translation initiation factors, IF1, IF2 and IF3, respectively. The molecular interactions involved in this process encompass base-pairing of the Shine and Dalgarno (SD) sequence on mRNA with the anti-SD sequence residing at the 3' end of 16S rRNA gene and the interaction of the start codon with the anticodon of fMet-tRNA\textsuperscript{Met}, respectively (Gualerzi & Pon, 1990; Gualerzi et al., 2001). In general, inefficient ribosome binding to the 5' ends of canonical mRNAs has been shown to decrease their stability (Wagner et al., 1994; Iost & Dreyfus, 1995; Arnold et al., 1998). Naturally occurring examples of mRNAs with a poor ribosome binding efficiency include leaderless mRNAs, which contain a 5' terminal start codon and lack canonical ribosome recruitment signals (Moll et al., 2002). One study (Baumeister et al., 1991) has demonstrated that the low translational efficiency of the leaderless Tn1721 tetR mRNA is associated with a short half-life.

As mentioned above, the decay of canonical mRNAs is frequently initiated by RNase E cleavages in the 5' UTR of mRNA (Melefors & von Gabain, 1988; Gross, 1991). RNase E cleaves A/U-rich regions (Lin-Chao et al., 1994; McDowall et al., 1994) that preferentially have a G positioned two nucleotides upstream from the scissile bond (Kaberdin, 2003; Redko et al., 2003). Such A/U-rich sequences are frequently found in the vicinity of the ribosome binding site (RBS) of E. coli mRNAs, and random cloning approaches have revealed that these sequences stimulate translation when placed up- (Dreyfus, 1988) or downstream (Qing et al., 2003) of a start codon. One rationale for these findings is that A/U-rich regions keep the translation initiation region unstructured and, thereby, facilitate ribosome binding. While interacting with the translation initiation region, the 30S ribosome subunit confers protection to an c. 50-nt region bracketing the start codon (Hüttenhofer & Noller, 1994). Hence, a ribosome bound at the RBS would be anticipated to protect A/U-rich regions flanking the start codon from RNase E cleavage. In support of this idea, in vitro experiments with E. coli ompA mRNA have shown that 30S ribosomal subunits bound to the RBS can protect from RNase E cleavage in the 5' UTR (Vytvytska et al., 2000). Moreover, mutations that extend the complementarity between Shine–Dalgarno (SD) and anti-SD sequences increased translation and mRNA stability in vivo (Wagner et al., 1994; Iost & Dreyfus, 1995; Arnold et al., 1998). Thus, it is tempting to speculate that a rate-limiting step in mRNA decay depends on the binding affinity of either the 30S ribosomal subunit or RNase E for a given mRNA.
A candidate for a ribosomal component that protects from RNase E cleavage is protein S1. This ribosomal protein is known to contribute to the 30S/mRNA interaction in E. coli (Boni et al., 1991; Komarova et al., 2005). Cryo-electron microscopic studies (Sengupta et al., 2001) revealed that S1 interacts with 11 nucleotides located immediately upstream of the SD sequence. S1 binding to this region is considered of importance for 30S ribosomal recognition of the 5′ region of E. coli mRNAs and/or for stabilization of the ternary complex (Boni et al., 1991). Experiments performed in our laboratory showed that S1 binding sites and RNase E cleavage sites can coincide on different mRNA substrates, suggesting that ribosome binding to the translation initiation region might involve protection of RNase E cleavage sites by S1. Proximal RNase E cleavage upstream of the RBS (i.e. within a S1 binding site) is not only expected to diminish or eventually prevent further ribosome loading, but would also generate a 5′ monophosphate group at the 5′ end of the processed mRNA, which in turn is expected to stimulate RNase E cleavages within the ribosome-free, downstream region (Fig. 1b, left). The location of coincident S1 binding and RNase E cleavage sites upstream of the SD sequence could provide a means to eliminate selectively mRNAs that are in a translationally ‘inactive conformation’, e.g. where the SD sequence and/or the start codon are sequestered by secondary structure. This would diminish ribosome loading, whereas RNase E cleavage could be unaffected. Moreover, when the concentration of free 30S ribosomal subunits becomes limiting, endonucleolytic cleavages at the 5′ end could provide a mechanism to redirect ribosomes to transcripts, the 5′ UTRs of which are intrinsically more resistant to the nucleolytic activity of RNase E.

Recent studies revealed that ribosome binding to the translation initiation region per se is not sufficient to protect the entire body of E. coli mRNAs from degradation (Arnold et al., 1998; Joyce & Dreyfus, 1998). Joyce & Dreyfus (1998) demonstrated that, in the absence of translation, lacZ mRNA was not stabilized by the presence of a long SD sequence at its 5′ end. Likewise, it has been recently found that ongoing translation rather than ribosome binding per se is required to protect an ompA-bla fusion mRNA from degradation (Arnold et al., 1998), and the stabilizing effect of ribosome traffic apparently depends on the length of translated segments of this transcript (Nilsson et al., 1987).

A recent study by the Boni group (Komarova et al., 2005) provided further support for these findings. The introduction of A/U-rich sequences upstream of the SD increased translation and the stability of lacZ mRNA, which can be reconciled with the idea that the creation of S1-binding sites accelerates the forward kinetics of ribosome binding and consequently coverage of the body of the mRNA by elongating 70S particles (Komarova et al., 2005). As further discussed below, mRNA decay in E. coli can thus be considered a consequence of translational inhibition, i.e. in the absence of ribosome traffic along the mRNA it becomes vulnerable to endonucleolytic attack (Fig. 1b, on the left).

In contrast to E. coli mRNAs, several mRNAs of Gram-positive bacteria such as B. subtilis phage SP82 RNA (Hue et al., 1995) and B. thuringiensis cryIIIA toxin RNA (Agaisse & Lereclus, 1996) have been proposed to be stabilized by either binding or stalling of ribosomes near the 5′ end. In both cases canonical SD-sequences function as stabilizers of the downstream segments, and ribosome binding might protect the 5′ ends of the transcripts from degradation by nucleases. In agreement, the phage SP82 stabilizer conferred increased stability to several heterologous mRNAs when inserted at their 5′ end (Hue et al., 1995). Further experiments demonstrated the importance of translation initiation complex formation at the 5′ end of mRNAs, rather than the transit of ribosomes along the mRNA in B. subtilis (Sharp & Bechhofer, 2003), leading the authors to suggest that ribosome binding to the 5′ end of the mRNA interferes with a 5′-end dependent activity, possibly a 5′-binding endonuclease such as RNase J1 and/or RNase J2 (Even et al., 2005).

Control of translation in Gram-positive bacteria can also involve leader peptides encoded by short ORFs often located immediately upstream of antibiotic resistance genes (e.g. Staphylococcus aureus ermA, ermC and cat mRNAs (Bechhofer, 1990; Dreher & Matzura, 1991; Bechhofer, 1993; Drider et al., 2002). In the absence of the antibiotic, the RBS for the resistance determinant is normally sequestered in a secondary structure domain within its cognate mRNA, thus inhibiting translation. However, ribosome stalling induced by subinhibitory concentrations of the antibiotic during translation of the leader peptide results in structural rearrangements that destabilize the inhibitory structure, and therefore allow ribosome binding and subsequent translation of the drug resistance genes, a mechanism known as translation attenuation. The antibiotic-induced stalling in the leader peptide coding sequence highly stabilizes the transcript (Bechhofer & Dubnau, 1987). Thus, ribosome stalling at the 5′-end of an mRNA appears to protect the downstream gene in the same manner as translation initiation complex formation, again suggesting a 5′ → 3′ directional stabilization, i.e. protection against a 5′-end dependent nuclease activity in Gram-positive organisms.

**Translational repression by proteins**

Several studies have shown that protein factors can inhibit translation by competing with ribosome binding and, thus, affect the longevity of an mRNA (Fig. 1b, right). In the following, we will only deal with a few examples in E. coli, where the interrelation between translational repression and mRNA degradation has been studied. Some of these
regulatory mechanisms involve molecular mimicry. For instance, while regulating their own production, some ribosomal proteins bind to the translational operators that are structurally similar to the regions in ribosomal RNA with which these proteins interact during ribosome assembly. Similarly, the translational operator in thrS mRNA, encoding threonyl-tRNA synthetase (ThrRS), has a tRNA-like structure (Springer et al., 1986).

Many ribosomal protein genes are organized in operons, and their synthesis is coordinated to ensure that they are produced in stoichiometric amounts. When in excess over ribosomal RNA, some ribosomal proteins function as translational repressors. Moreover, they inhibit the synthesis of other ribosomal proteins encoded within the same polycistrionic transcripts (Keener & Nomura, 1996). In most, if not all, cases this is achieved by positive translational coupling, whereby translation of the downstream gene requires translation of the preceding region. In other words, if translation of the upstream gene is blocked by translational repression, translation of the downstream genes ceases as well. Repressor binding and subsequent inhibition of translation destabilizes several polycistrionic mRNAs coding for ribosomal proteins. Examples include the α operon encoding ribosomal proteins S13, S11, S4, the α-subunit of RNA polymerase and ribosomal protein L17. S4 acts as a translational repressor that inhibits synthesis of S13, S11 and S4 (but not L17) by binding to the 5′ UTR of the S13 gene (Singer & Nomura, 1985). Consequently, the stability of the mRNA segment that encodes S13, S11 and S4 decreases upon repression of S13 synthesis. Translational repression by ribosomal proteins can sometimes affect the stability of segments distant from as well as close to the translational operator. The spc operon encodes 10 ribosomal proteins including S8, which directly and indirectly (by impairing translational coupling) blocks synthesis of the third gene product L5 and several distal gene products, respectively. In addition, S8 mediated silencing of L5 translation regulates the synthesis of the first and second gene product, L14 and L24. The Nomura laboratory has shown that translational repression by S8 causes decay of the 5′ proximal portion of the mRNA, and that the two major E. coli exonucleases, PNPase and RNase II, are involved in this process (Mattheakis et al., 1989). The authors discussed their observations in terms of a model that included initial endonucleolytic cleavage downstream of the translational operator, followed by exonucleolytic 3′ → 5′ decay by PNPase and RNase II. A remaining question is how the exonucleolytic activities counteract the 5′ → 3′ transit of translating ribosomes in the proximal part of the spc mRNA.

The thrS gene encoding E. coli threonyl-tRNA synthetase (ThrRS) is autogenously controlled at the level of translation initiation. The dimeric ThrRS binds to two stem–loop structures upstream of the thrS start codon and its binding site does not strictly overlap the ribosome binding site (Romby & Springer, 2003). Recent crystallographic studies support an indirect competition mechanism in which the elongated N-terminal domain of ThrRS that is bound to the SD-proximal stem–loop structure sterically obstructs ribosome binding (Jenner et al., 2005). Therefore, translational repression by ThrRS appears to be governed kinetically through faster binding of ThrRS than the 30S subunit. The half-life of thrS mRNA is independent of the steady-state levels, suggesting that thrS mRNA is either translated or immediately degraded upon repressor binding (Nogueira et al., 2001). The stability of thrS mRNA is enhanced in a strain lacking functional RNase E, and small RNA fragments corresponding to the 5′ end of the mRNA accumulated in a strain lacking PNPase. Taken together, these studies suggest that endonucleolytic cleavage by RNase E is the rate-limiting step in thrS mRNA degradation, which is followed by 3′ → 5′ exonucleolytic trimming (Nogueira et al., 2001).

The phylogenetically conserved bacterial RNA chaperone Hfq mediates the interaction between ncRNAs (see below) and their mRNA targets (Valentin-Hansen et al., 2004). Hfq binds to two sites in the 5′-untranslated region of hfq mRNA and inhibits formation of the translation initiation complex (Veˇcerek et al., 2005). The steady state level of hfq mRNA depends on RNase E (Tsui et al., 1994). Although the levels of RNase E were reportedly unchanged in a strain carrying an insertion in the hfq gene, the half-life of hfq mRNA was lower in the presence of functional Hfq than in its absence (Tsui et al., 1997). Autogenous repression by Hfq would prevent subsequent transit of ribosomes through the coding sequence and would consequently render the untranslated mRNA vulnerable to RNase E cleavage.

**Control by thermo-responsive RNA structures**

As the equilibrium between alternative RNA structures with different thermodynamic stabilities is highly affected by temperature, different RNA folds can function as thermo-responsive elements that control translation efficiency as well as the stability of the corresponding mRNAs (Fig. 2a). Recent studies revealed that this type of regulation is employed by several microorganisms during adaptation to heat- or cold-shock (Gualerzi et al., 2003; Narberhaus et al., 2006).

Bacterial acclimatization to heat shock is largely dependent on an increase in the level of RpoH, also known as σ 32, σ factor that is required for efficient transcription of heat-shock genes. The translation initiation region of E. coli rpoH mRNA is occluded by a stable secondary structure at 30 °C, which is destabilized during heat shock (Morita et al., 1999a,b). The destabilizing effect of temperature on this structure in turn facilitates rpoH translation (Morita et al.,...
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Fig. 2. Regulatory roles of alternative structures at the 5′-end of bacterial mRNAs. (a) The equilibrium between alternative RNA structures that differ in energy is temperature-dependent. Consequently, temperature shifts can differentially affect the accessibility of translation initiation regions (TIR) and thereby influence the fate of transcripts by increasing/decreasing their translation and stability. (b) Alternative RNA structures induced by low molecular weight metabolites. Depending on the nature of each particular riboswitch, its interaction with an effector (red rhombs) such as amino acids, nucleotides, vitamins can either activate (upper panel; Mandal & Breaker, 2004) or inhibit (lower panel; Miranda-Rios et al., 2001; Mironov et al., 2002; McDaniel et al., 2003) translation of the downstream gene, which in turn can lead to stabilization or degradation of the corresponding mRNA(s), respectively.

Based on the observation that the steady-state level of rpoH mRNA increased eightfold when cells are shifted from 30 to 42 °C but rpoH mRNA synthesis increased only by twofold, it has been proposed that the rpoH mRNA level is posttranscriptionally regulated (Erickson et al., 1987), presumably through protection from nucleases by translating ribosomes.

Similar regulatory circuits operate in Rhizobia. However, in addition to RpoH-dependent mechanisms, some heat-shock operons are under control of a conserved regulatory element termed ROSE (repression of heat shock gene expression) that acts as a thermosensor. This motif is generally located in the 5′ UTR. Under normal conditions, ROSE elements repress downstream genes’ expression by adopting secondary structures that occlude their translation initiation regions. Derepression of translation occurs through structural rearrangements within the 5′ UTR upon temperature up-shifts, thus favouring ribosome binding and subsequent translation of the ROSE-controlled genes under heat-shock conditions. Interestingly, temperature-induced ribosome binding and consequently translation seems to protect ROSE mRNA(s) against ribonucleolytic degradation, thereby resulting in increased levels of these transcripts at elevated temperatures (Nocker et al., 2001).

Exposure of bacterial cells to a significant temperature downshift induces the cold-shock response that leads to increased production of cold-shock proteins (Thieringer et al., 1998; Gualerzi et al., 2003). The mRNA encoding the major cold-shock protein, CspA, is transcribed at 37 °C but is hardly detectable due to efficient degradation by RNase E (Brandi et al., 1996; Fang et al., 1997). However, the same transcript is stabilized more than 100-fold upon temperature downshift to 10 °C (Brandi et al., 1996; Fang et al., 1997). The stability determinants are located in the 5′ UTR of this transcript (Fang et al., 1997). Furthermore, the 5′ UTR of cspA mRNA appears to be more important for efficient translation of cspA mRNA under cold shock than at 37 °C (Giuliodori et al., 2004). Nonetheless, it remains to be shown whether the mechanisms controlling the stability and translation of cspA mRNA are interrelated and whether they are linked to some structural rearrangements.

Conformational changes in RNA might also play an important role during less profound temperature shifts (5–10 °C), apparently to balance the level of individual proteins. For instance, while studying the effect of temperature on the stability and translation of ompA mRNA, we have recently found that the concentration of ompA mRNA is twofold lower at 37 °C than at 28 °C, whereas the amount of OmpA is maintained more or less constant at both temperatures (Afonyushkin et al., 2003). This suggested that the decrease in the steady-state level of mRNA at 37 °C is compensated by a higher translation rate. A possible mechanism contributing to the temperature-dependent stability and ompA mRNA translation seems to involve structural changes in the 5′ UTR of the transcript. A number of alternative structures have been suggested previously, and it was proposed (Rosenbaum et al., 1993) that some of these coexisting structures might occlude the RBS and/or modulate susceptibility to RNase E. It is thus conceivable that temperature regulates the stability and translation of ompA mRNA (and presumably other transcripts) by changing the ratio between alternative structures within its 5′ UTR.

Last but not least, there is evidence that RNA thermosensors are involved in regulating the production of virulence factors in pathogenic bacteria (Hoe & Goguen, 1993; Johansson et al., 2002). Although their role in translation activation is well established, very little is known how these...
regulatory elements affect the stability of the corresponding RNAs.

**Low molecular weight effectors and riboswitches**

An increasing number of low molecular weight effectors such as amino acids, coenzymes or vitamins have been recently found to bind to the 5'-UTR of many mRNAs to regulate their function (Fig. 2b). The structural elements responsible for effector binding and subsequent changes in the expression pattern of their cognate mRNAs are referred to as ‘riboswitches’ (Tucker & Breaker, 2005; Winkler & Breaker, 2005). One of the alternative conformations adopted by a common riboswitch in the presence/absence of molecular effectors frequently contains an embedded hairpin structure that causes transcription termination (Mironov et al., 2002; Winkler et al., 2002a, b; Epshtein et al., 2003). Although this type of regulation is known to occur in several cases, riboswitches can also exert their regulatory functions at the posttranscriptional level (Nou & Kadner, 1998; Vitreschak et al., 2002a, b). For instance, structural rearrangements that occur upon effector binding to or upon dissociation from its cognate RNA element can result in a stem–loop structure that sequesters the translation initiation region (Fig. 2b) (Nou & Kadner, 1998; Vitreschak et al., 2002; Winkler et al., 2002a, b). As exemplified for the E. coli *btu* mRNA, encoding the outer membrane cobalamine transporter BtuB, such structural changes can affect the stability of the riboswitch-regulated transcript (Nou & Kadner, 1998). Analysis of a *btu-lacZ* transcript demonstrated that translation inhibition by adenosylcobalamin decreases its steady-state level, which is possibly due to increased vulnerability of the ribosome-free mRNA to RNases (Nou & Kadner, 1998). Future studies will reveal whether the same or different mechanisms operate in other cases.

**Antisense control by cis- and trans-encoded RNAs**

Posttranscriptional mechanisms in bacteria often involve the action of *cis*- and *trans*-encoded antisense RNAs (Carpousis, 2003; Gottesman, 2005). Most of these small, noncoding RNAs (ncRNAs) (Fig. 3), including FinP (van Biesen et al., 1993), Sok (Gerdes et al., 1992), RyhB (Massé et al., 2003; Geissmann & Touati, 2004; Afonyushkin et al., 2005), MicA (Rasmussen et al., 2005; Udekwu et al., 2005) and MicF (Delihas & Forst, 2001), basepair with the translation initiation region of their target mRNAs, and the resulting...
inhibition of translation leads to degradation of the ribosome-free mRNAs by the concerted action of endo- and exoribonucleases (Fig. 4a).

Cis-encoded antisense RNAs that originate by convergent transcription from the same genetic locus as the target RNA(s) control many biological functions including plasmid replication, maintenance and transfer, the lysis/lysogeny decisions of bacteriophages, and the transposition as well as regulation of plasmid and host toxin–antitoxin systems (Fig. 3a) (Simons & Kleckner, 1988; Wagner et al., 2002). Although this class of regulatory RNAs is widespread in bacterial accessory genetic elements, comparatively little is known about antisense RNAs encoded by bacterial genomes. The control of numerous host- and plasmid-encoded toxin–antitoxin systems is tightly regulated by cis-acting antisense RNAs that are complementary to the leader regions of the corresponding target mRNAs encoding for various toxins (Gerdes et al., 1997). Previous work on the hok/sok system from plasmid R1 revealed that, while basepairing with the translation initiation region of the hok mRNA, Sok antisense RNA inhibits translation, and consequently targets hok mRNA for RNase III-mediated degradation (Gerdes et al., 1992). Thereby the level of Hok protein, a bacterial toxin damaging the cell membrane, is minimized. Furthermore, RNase III has been shown to be involved in the initial decay of a number of inhibitory structures that are formed upon basepairing of other antisense RNAs with their targets. These structures include the Tn10 transposase mRNA/OOP RNA duplex (Krinke & Wulff, 1987; Krinke & Wulff, 1990a, b) and apparently the traf mRNA/FinP duplex of the FinOP fertility inhibition system of F-like plasmids (Jerome et al., 1999). It is of interest that, similar to the action of the E. coli RNA chaperone Hfq (see below), some RNA-binding proteins [such as the E. coli RNA chaperone FinO (Arthur et al., 2003)] can protect cis-encoded antisense RNAs from RNase E cleavage in vivo and facilitate sense-antisense RNA interactions (Jerome et al., 1999).

In contrast to cis-encoded antisense RNAs, the trans-encoded antisense RNAs or riboregulators (Fig. 3b) are encoded at loci other than those of their target genes. Many of these riboregulators can affect multiple targets, and their level is increased in response to environmental cues, such as nutrient depletion, changes in pH, temperature shifts, etc. (Storz & Hengge-Aronis, 2000). In general, these ncRNAs display only partial complementarity to their target RNAs and require the RNA-binding protein [such as the RNA chaperone Hfq (see below)], some ncRNAs [such as the E. coli RNA chaperone FinO (Arthur et al., 2003)] can protect cis-encoded antisense RNAs from RNase E cleavage in vivo and facilitate sense-antisense RNA interactions (Jerome et al., 1999).
The RNA-chaperone activity of Hfq caused local structural changes in the ompA 5' UTR (Moll et al., 2003a,b), which prevented ribosome binding in vitro (Vytvytska et al., 2000). Nonetheless, recent work suggests that Hfq may not exert its regulatory function alone but in combination with ncRNAs. At least two trans-encoded regulatory RNAs, MicA (Rasmussen et al., 2005; Udekwu et al., 2005) and RseX (Douchin et al., 2006), are able to basepair with the ompA 5' UTR. They apparently prevent ribosome binding and consequently destabilize the entire ompA mRNA (Rasmussen et al., 2005; Udekwu et al., 2005; Douchin et al., 2006).

Recent studies of the posttranscriptional mechanism regulating the metabolic stability of E. coli ptsG mRNA in response to accumulation of glucose-phosphate added a new twist to ncRNA-mediated antisense control. The work revealed that basepairing between this mRNA and the ncRNA SgrS was more efficient when ptsG mRNA was localized near the membrane (Kawamoto et al., 2005). Kawamoto et al. (2005) suggested that cotranslational targeting of ptsG mRNA to the membrane affects competition between ribosomes and SgrS for access to the 5' UTR of this transcript (Kawamoto et al., 2005; Vanderpool & Gottesman, 2005) and, therefore, plays an important regulatory role.

Staphylococcus aureus RNAIII is one of the largest regulatory RNAs (514 nt), which plays an essential role in the coordination of virulence gene expression in response to various environmental signals (Novick & Jiang, 2003). Recent work has shown that RNAIII basepairs with the 5' UTR of spa mRNA, encoding for the surface protein A, and inhibits ribosome binding to the translation initiation regions of this transcript (Huntzinger et al., 2005). The basepairing seems to be facilitated by S. aureus Hfq and subsequently targets the spa mRNA for RNase III-mediated degradation (Huntzinger et al., 2005).

One of the best-characterized mechanisms underlying the functional inactivation of a target mRNA by a trans-encoded ncRNA is the RyhB-mediated decay of the E. coli sodB mRNA, encoding a superoxide dismutase (Massé & Gottesman, 2002; Massé et al., 2003; Geissmann & Touati, 2004; Afonyushkin et al., 2005). Recent work has shown that Hfq binding induces structural changes in the sodB translation initiation region, which seems to prime the mRNA for basepairing with RyhB (Geissmann & Touati, 2004), which in turn prevents ribosome binding (Večerek et al., 2003; Geissmann & Touati, 2004). Moreover, the structural rearrangements imposed in the sodB 5'-untranslated region upon basepairing with RyhB create new RNase III and RNase E cleavage sites in the ncRNA and its target mRNA, respectively (see Fig. 4a) (Afonyushkin et al., 2005). These data supported the idea of coupled degradation of ncRNAs and their mRNA targets originally proposed by Massé et al. (2003).

It is interesting to note that some trans-encoded riboregulators can functionally activate their target mRNAs. For example, DsrA and presumably RprA (Repoila et al., 2003) form a complex with their target mRNA, rpoS, that leads to an exoposition of the translation initiation determinants, and thereby facilitate translation (Fig. 4b) (Lease et al., 1998; Majdalani et al., 1998, 2001). As a result, the translationally active form of rpoS mRNA is stabilized (Lease & Belfort, 2000a,b).

Alba and coworkers have recently obtained evidence for a direct interaction of Hfq with RNase E (Morita et al., 2005). These authors devised a model which specified how RNase E is targeted to ncRNA/mRNA complexes and explained the previously observed RNase E-dependent degradation of ncRNA targets (Massé et al., 2003; Kawamoto et al., 2005; Vanderpool & Gottesman, 2005). The Alba group further showed that a decrease in RNase E activity per se had no significant effect on translational inhibition by trans-encoded RNAs in vivo (Morita et al., 2006), which supported the idea that this enzyme is mainly involved in the decay of translationally inactive mRNAs. Although endonucleolytic cleavages within some ncRNA/mRNA duplexes may play the key role in generation of the primary intermediates that are further targeted for RNase E-dependent decay, it remains questionable whether they are generated by RNase E, known to be a single-strand specific endoribonuclease. Instead, an increasing number of posttranscriptional mechanisms that are controlled by cis-encoded (Simons & Kleckner, 1988; Blomberg et al., 1990; Krinke & Wulff, 1990a,b; Gerdes et al., 1992; Jerome et al., 1999) and some trans-encoded (Vogel et al., 2004; Afonyushkin et al., 2005; Huntzinger et al., 2005) riboregulators, suggest that this role can be assigned to RNase III. Consequently, further work is needed to clarify, whether RNase E or RNase III cleavage(s) is rate-limiting in ncRNA-mediated decay of mRNA.

**Perspectives**

Although information on key players of the E. coli RNA degradation and protein-synthesizing machineries has increased tremendously over the past decade, the coordination of their activities to regulate the fate of individual mRNAs has been addressed in detail only for a limited number of transcripts. New challenges in the field arise with regard to the posttranscriptional mechanisms controlled by the plethora of ncRNAs, low molecular weight effectors and temperature shifts. It remains to be seen whether the formation of ncRNA/mRNA complexes or alternative RNA structures generally lead to the creation of new RNase III/RNase E-like sites, and which of the enzymes is in charge of the rate-limiting step in disassembly and processing of the intermediate regulatory structures and complexes. In the era of systems biology, the elucidation of complex and diverse
posttranscriptional control mechanisms by linking translation and mRNA decay certainly becomes an important step towards understanding the global regulatory networks that coordinate physiological adjustments in microorganisms.

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References


Cannistraro VJ & Kennell D (1985) Evidence that the 5’ end of lac mRNA starts to decay as soon as it is synthesized. J Bacteriol 161: 820–822.


Krinke L & Wulff DL (1987) OOP RNA, produced from multicopy plasmids, inhibits lambda cII gene expression


Vanderpool CK & Gottesman S (2004) Involvement of a novel transcriptional activator and small RNA in post-


