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Posttranscription Initiation Control of Gene Expression Mediated by Bacterial RNA-Binding Proteins

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Abstract

RNA-binding proteins play vital roles in regulating gene expression and cellular physiology in all organisms. Bacterial RNA-binding proteins can regulate transcription termination via attenuation or antitermination mechanisms, while others can repress or activate translation initiation by affecting ribosome binding. The RNA targets for these proteins include short repeated sequences, longer single-stranded sequences, RNA secondary or tertiary structure, and a combination of these features. The activity of these proteins can be influenced by binding of metabolites, small RNAs, or other proteins, as well as by phosphorylation events. Some of these proteins regulate specific genes, while others function as global regulators. As the regulatory mechanisms, components, targets, and signaling circuitry surrounding RNA-binding proteins have become better understood, in part through rapid advances provided by systems approaches, a sense of the true nature of biological complexity is becoming apparent, which we attempt to capture for the reader of this review.

Keywords

RNA-binding protein; gene regulation; attenuation; antitermination; translation; sRNA

INTRODUCTION

Bacteria have the capacity to rapidly alter metabolism, physiology, behavior, and morphology in response to changing environmental conditions by altering gene expression. Although transcription initiation plays a vital role in response to environmental stimuli, regulation that occurs after transcription initiation is now recognized as a common regulatory strategy in all organisms. A wide variety of posttranscriptional regulatory mechanisms have been identified, including regulated transcription termination (attenuation and antitermination), regulation of translation initiation, and changes in mRNA stability. The

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factors responsible for sensing and responding to changing environmental conditions include RNA-binding proteins and small RNAs (sRNAs). This review focuses on mechanisms that RNA-binding proteins utilize to control bacterial gene expression, although the role of sRNAs that affect the activity of certain RNA-binding proteins will also be discussed. Excellent reviews describing enzymes involved in mRNA turnover, as well as the mechanism by which the RNA chaperone Hfq mediates sRNA-mRNA base-pairing, have been published recently, and these are not reviewed here (38, 72, 88, 116).

Once transcription initiates, the transcription elongation complex is subject to regulatory events including RNA polymerase pausing and transcription termination. These processes are modulated by the general transcription elongation factors NusA and NusG (23, 90, 152). During transcription the nascent transcript can adopt alternative antiterminator and terminator structures that dictate whether RNA polymerase continues to transcribe into the downstream protein-coding sequences, or whether transcription terminates in the 5′ untranslated region (5′ UTR). NusA and NusG can stimulate RNA polymerase pausing, which can synchronize RNA polymerase position with RNA folding and/or binding of a regulatory factor to the nascent transcript (23, 152, 155). Transcription attenuation is defined as promotion of transcription termination in the 5′ UTR by the action of a regulatory molecule (translating ribosome, RNA-binding protein, RNA, metabolite), with the default being transcription read-through into the downstream structural gene(s). Antitermination is distinguished from attenuation in that the action of the regulatory molecule results in transcription read-through, with the default being termination (47). Mechanisms involving the RNA-binding proteins HutP and the Bgl-Sac family of proteins of *Bacillus subtilis* and *Escherichia coli* are used to illustrate well-characterized antitermination mechanisms, while the actions of *trp* RNA–binding attenuation protein (TRAP) and PyrR of *B. subtilis* are used to describe transcription attenuation.

RNA-binding proteins are also capable of regulating translation initiation. Although the vast majority of the known regulatory mechanisms repress translation initiation, a few examples of translational activation have been identified (14). RNA-binding proteins can promote the formation of alternative RNA structures that dictate the accessibility of 30S ribosomal subunits to the Shine-Dalgarno (SD) sequence, a critical component of the ribosome-binding site. Sequestration of the SD sequence in RNA structure represses translation, whereas destabilization of such structures can activate translation. Translation can also be repressed directly such that the RNA-binding protein directly occludes ribosome binding. Mechanisms of RNA-binding-protein-mediated translational control are illustrated by descriptions of TRAP, CsrA, and the *E. coli* cold shock protein CspA.

RNA-binding proteins throughout the biological world interact with noncoding RNAs that regulate gene expression either by altering the activity of the RNA-binding protein itself or by influencing the formation of sRNA-mRNA base-pairing interactions. The three-factor interactions thus generated can create diverse regulatory effects and patterns. The CsrA/RsmA family of bacterial proteins provides an example of RNA-binding proteins whose activity is regulated by binding to sRNAs containing many high-affinity CsrA-binding sites, which decrease the availability of these proteins to interact with mRNA targets with lower binding affinity. The global regulatory roles of CsrA/RsmA proteins and their sRNAs have

attracted widespread attention (112, 143). A second RNA-binding protein, RapZ, interacts with two related sRNAs, affecting the base-pairing activity of one of these sRNAs while being sequestered and inhibited by the second sRNA, which acts as a decoy (50). The FinO/ProQ family of RNA-binding proteins offers a final example of protein-sRNA-mRNA interactions, only recently recognized for potentially diverse and pervasive regulatory roles (60, 95).

REGULATED TRANSCRIPTION TERMINATION: ATTENUATION AND ANTITERMINATION

Bgl-Sac Family of Antitermination Proteins of *B. subtilis* and *E. coli*

A common antitermination mechanism controls expression of genes involved in the utilization of β-glucosides, sucrose, and glucose in *E. coli* and/or *B. subtilis* (3, 129). The general antitermination mechanism was first described for BglG-mediated regulation of the *E. coli bglGFBH* operon (4, 5). Subsequent studies of the SacT, SacY, LicT, and GlcT antitermination proteins from *B. subtilis* contributed greatly to our understanding of this fascinating mechanism (e.g., 9, 20, 122, 131, 135, 136). Each antitermination protein consists of an N-terminal RNA-binding domain and two regulatory domains called PRD1 and PRD2. These regulatory domains are the sites of reversible phosphorylation at conserved histidine residues in response to the cognate carbon source (6, 84, 130). In the absence of the carbon source, these proteins are rendered inactive by phosphorylation of PRD1 by the cognate membrane-bound EII transporter of the phosphotransferase system (PTS) (54, 78). In the presence of the carbon source the EII transporter dephosphorylates PRD1, while HPr, a component of the PTS that is sugar-nonspecific, phosphorylates PRD2 (54, 78, 115, 130). When PRD1 is unphosphorylated and PRD2 is phosphorylated the antitermination protein forms an active homodimer (5, 115, 121). The active dimer then binds to its target transcripts and stabilizes an otherwise weak antiterminator structure in the 5′ UTR termed RAT, for RNA antiterminator (5, 12). As a consequence, an overlapping intrinsic terminator is unable to form, leading to transcription of the operon. In the absence of bound protein, the terminator hairpin forms and transcription halts upstream of the coding sequence (Figure 1). One notable exception to this antitermination mechanism is the apparent absence of an intrinsic terminator that overlaps the RAT in the 5′ UTR of the *sacXY* operon (136). The mechanism by which this operon is regulated by SacT and SacY has not been elucidated.

Structural studies from several Bgl-Sac family proteins confirmed that the RNA-binding domain forms a symmetrical homodimer, with each monomer forming a four-stranded β sheet (32, 84, 163). A solution structure of the LicT RNA-binding domain in complex with its cognate RAT showed that LicT interacts with two bulges within the RAT, and with the minor groove of the stem between these two loops (163). Despite their sequence and structural similarity, these antitermination proteins are highly specific for their cognate RNA targets. Substitution of residues in SacT, LicT, and SacY that contact their cognate RATs resulted in reduced binding specificity (61). A crucial arginine residue in LicT was also identified that is required for high affinity and binding specificity. Interestingly, introduction of this residue in SacY increased its RNA-binding affinity but reduced its specificity for its

cognate RNA target. These results led to the suggestion that this family of antitermination proteins evolved to maintain specificity by compromising binding affinity for their cognate RNA targets (61).

HutP-Mediated Antitermination in *B. subtilis*

The *B. subtilis hutPHUIGM* operon encodes an RNA-binding protein that regulates expression of the operon (HutP), a histidine transporter (HisM), and enzymes for the utilization of histidine as a carbon and nitrogen source (73). HutP regulates expression of the *hut* operon by an antitermination mechanism in response to histidine. Under low histidine, an intrinsic terminator causes transcription to terminate in the *hutP-hutH* intercistronic region (150). However, under histidine excess, histidine-activated HutP binds to the nascent *hut* transcript and prevents formation of the intrinsic terminator such that transcription continues into the downstream genes (53, 93, 94). Thus, antitermination allows increased transport and utilization of histidine as a carbon and nitrogen source (Figure 2).

HutP functions as a homohexamer, and binding of both histidine and Mg^{2+} is required to activate its RNA-binding activity (94). A crystal structure of HutP demonstrated that each monomer consists of a four-stranded antiparallel β sheet, with two α helices located on each side of the β sheet (76). A structure of the activated HutP-histidine-Mg^{2+} complex demonstrated that histidine and Mg^{2+} interact with one another, that histidine contacts several amino acid residues in HutP, and that six molecules of both histidine and Mg^{2+} bind to each HutP hexamer (76). When activated by histidine and Mg^{2+}, HutP binds to NAG triplet repeats that are separated by two to four nonconserved spacer residues (74, 93). A crystal structure of activated HutP in complex with an RNA containing three UAG repeats revealed that one RNA molecule bound to one surface of HutP and a second RNA molecule bound to an equivalent surface on the opposite side of HutP. The bound RNA molecules adopted a triangular shape on each HutP RNA-binding surface (75). The natural RNA target in the *hutP-hutH* intercistronic region contains two clusters of three NAG repeats, with the two clusters separated by 20 nucleotides. A crystal structure of HutP with an RNA molecule containing six UAG repeats that mimicked the natural RNA target demonstrated that one cluster bound to each HutP surface, with the long spacer separating each cluster wrapping around one side of the HutP hexamer (76).

PyrR-Mediated Attenuation in *B. subtilis*

The *B. subtilis pyrRPBC-AA-AB-KDFE* operon encodes an RNA-binding protein that regulates expression of the operon (PyrR), a uracil transporter (PyrP), and the enzymes for de novo biosynthesis of UMP (137). PyrR functions as a homodimer and is responsible for sensing both UMP and UTP in the cell (26, 81). Whereas UMP and UTP activate PyrR to bind to its RNA targets, GMP, GDP, and GTP bind to the same allosteric site of PyrR and compete with UMP/UTP binding (26, 29, 68).

PyrR regulates expression of the *pyr* operon by three essentially identical transcription attenuation mechanisms, each involving overlapping antiterminator and terminator structures (Figure 3). These attenuators are located in the *pyr* operon 5′ UTR, as well as in the intercistronic regions between *pyrR* and *pyrP* and between *pyrP* and *pyrB* (82, 83, 139).

In each case, UMP-activated PyrR binds to an RNA structure called the binding loop. Since the binding loop includes nucleotides that are required for antiterminator formation, bound PyrR favors termination (82, 83, 139). Thus, the binding loop functions as an anti-antiterminator when bound by PyrR. Each of the three PyrR binding loops contains an identical ARUCCAGAGAGGYU sequence with the underlined residues residing in the loop of the hairpin. In addition to this binding element, a conserved sequence/structural motif is present closer to the base of the binding loop. Mutagenesis studies demonstrated that substitution of several of the residues in these two RNA elements inhibited PyrR binding (26). Mutational studies of PyrR further revealed that a basic concave surface is involved in RNA recognition (119). Further details of PyrR-RNA interaction will require a structure of this complex.

NusA is a general transcription elongation factor that binds to RNA polymerase soon after sigma subunit release. RNA polymerase pausing appears to play a role in the three PyrR attenuation mechanisms. NusA stimulates pausing in vitro at positions that are present within the 3′ side of all three antiterminator structures (166). Each of these pause sites is positioned such that it could provide additional time for PyrR binding.

In addition to regulating *pyr* operon expression, PyrR is capable of catalyzing the uracil phosphoribosyltransferase (UPRTase) reaction involved in the uracil salvage pathway. However, the affinity for uracil is much lower than that of the UPRTase activity encoded by *upp*, suggesting that PyrR does not play a significant role in this biochemical pathway (134, 138). Rather, it was proposed that the UPRTase activity may have been its original function prior to evolving its RNA-binding activity (137).

TRAP-Mediated Attenuation in *B. subtilis*

The *B. subtilis trpEDCFBA* operon and *trpG* (*pabA*) encode the enzymes to convert chorismic acid, the common aromatic amino acid branch point, to tryptophan. *mtrB* encodes TRAP of *B. subtilis*. TRAP is composed of 11 identical subunits arranged in a single ring and is responsible for regulating tryptophan biosynthesis and transport in response to tryptophan (13). Tryptophan activates TRAP by binding between each adjacent subunit. Nine hydrogen bonds are formed between tryptophan and amino acids residing on adjacent subunits, with the indole ring buried in a hydrophobic pocket (7, 8). NMR studies indicate that tryptophan binding causes a structural change that is required for high-affinity TRAP-RNA interaction (86). Tryptophan-activated TRAP binds to NAG triplet repeats (GAG>UAG>AAG>CAG), with each repeat typically separated by two or three nonconserved residues (15, 17, 19). A crystal structure of the TRAP-RNA complex demonstrated that the RNA wraps around the perimeter of the TRAP ring and that each triplet repeat interacts with a KKR motif on the TRAP perimeter (7).

TRAP regulates expression of the *trp* operon by a transcription attenuation mechanism in which mutually exclusive antiterminator and terminator structures can form in the nascent *trp* transcript (Figure 4a). Under limiting tryptophan, TRAP is not activated and does not bind to RNA. In this case the antiterminator forms in the 5′ UTR such that transcription proceeds into the *trp* operon structural genes (18, 96). Under excess tryptophan, tryptophan-activated TRAP binds to 11 triplet repeats in the RNA, 7 of which are present within

the sequence that forms the antiterminator structure. As a consequence, bound TRAP promotes termination in the 5′ UTR by preventing formation of the antiterminator (18). The general transcription elongation factors NusA and NusG stimulate pausing just upstream of the critical overlap between the antiterminator and terminator structures. Pausing at this position provides additional time for TRAP to bind to the nascent transcript in vitro, although pausing at this position has not been substantiated in vivo (89, 151, 156). TRAP also contributes to termination in the 5′ UTR by a mechanism that is independent of its role in preventing formation of the antiterminator. Although the mechanism has not been firmly established, it appears that bound TRAP promotes forward translocation of RNA polymerase, thereby increasing the termination efficiency (85, 105).

In addition to the antiterminator and terminator structures, a 5′ stem-loop (5′ SL) structure that forms just upstream of the triplet-repeat region participates in the attenuation mechanism (35). Not only does TRAP-5′ SL interaction increase the affinity of TRAP for *trp* leader RNA, but this interaction also orients the downstream triplet repeats for interaction with the 11 KKR motifs that lie on the TRAP perimeter, thus increasing the likelihood that TRAP will bind in time to promote termination (87).

Another protein called anti-TRAP functions as a TRAP antagonist by binding to tryptophan-activated TRAP (144, 145). Binding of one trimeric anti-TRAP protein is sufficient to prevent TRAP-RNA interaction (124). Expression of *rtpA*, the gene encoding anti-TRAP, responds to the accumulation of uncharged $tRNA^{Trp}$ via the T box riboswitch mechanism (56, 118). Thus, *B. subtilis* controls tryptophan metabolism by sensing the levels of both free tryptophan and uncharged $tRNA^{Trp}$.

REGULATION OF TRANSLATION INITIATION

TRAP-Mediated Translational Repression in *B. subtilis*

In addition to regulating expression of the *trpEDCFBA* operon by transcription attenuation, TRAP regulates translation initiation of *trpE* (Figure 4a). In the absence of bound TRAP, *trp* operon read-through transcripts adopt an RNA secondary structure in which the *trpE* SD sequence is available for ribosome binding. However, when TRAP is bound to a read-through transcript, an RNA structure forms that sequesters the *trpE* SD sequence such that ribosome binding is inhibited (33, 117). A NusG-dependent RNA polymerase pause site located 3–4 nucleotides downstream from the 5′ UTR termination sites participates in this translation control mechanism by providing additional time for TRAP binding (154, 155, 156). NusG-dependent pausing depends on sequence-specific contacts with unpaired T residues in the nontemplate DNA strand within the paused transcription bubble (154). Because the *trpE* and *trpD* coding regions overlap by 29 nucleotides, TRAP-dependent translational repression of *trpE* is extended to *trpD* via translational coupling, a process in which translation of the downstream cistron is at least partially dependent on translation of the cistron just upstream. In addition, TRAP-dependent formation of the *trpE* SD–sequestering hairpin allows Rho access to the nascent transcript, causing transcriptional polarity (158).

TRAP also represses translation initiation of the unlinked *trpG* (*pabA*) (34, 162), *trpP* (118, 161), and *ycbK* (160) genes in response to tryptophan (Figure 4b). *trpG* is the second gene in an operon primarily concerned with folate biosynthesis, *trpP* (tryptophan transport) is a single-gene operon, while *ycbK* (unknown function) is in the *rtpA-ycbK* operon; recall that *rtpA* encodes anti-TRAP. TRAP binds to a triplet-repeat region that overlaps the cognate SD sequence and translation initiation regions of *trpG*, *trpP*, and *ycbK*, directly occluding ribosome binding in each case (34, 160, 161). Thus, TRAP coordinately regulates tryptophan biosynthesis and transport using three distinct mechanisms: by transcription attenuation of the *trpEDCFBA* operon, by promoting formation of the *trpE* SD-sequestering hairpin, and by directly blocking ribosome access to the *trpG* and *trpP* SD sequences.

CspA-Mediated Cold Shock Response in *E. coli*

When *E. coli* cells growing at 37°C experience a temperature downshift to 10°C, cell growth enters a lag phase for approximately 4 h. During this cold shock acclimation phase, the accumulation of 17 cold shock–induced proteins was demonstrated by 2D gel electrophoresis (67). The major cold shock protein is CspA, constituting approximately 13% of the total cellular protein synthesis during acclimation (46). CspA acts as an RNA chaperone that destabilizes global mRNA structure at low temperatures by binding preferentially to pyrimidine-rich RNA sequences (66). Bound CspA destabilizes secondary structures to promote translation or alter mRNA turnover of downstream genes.

CspA is structurally related to the cold shock domain of Y-box protein in eukaryotes, which forms a β-barrel structure with a positively charged RNA-binding surface (120). Three aromatic amino acids on the RNA-binding surface promote binding and destabilization of the RNA secondary structure. Base-stacking interactions between these aromatic amino acids and nucleotide bases in the apical loop of a hairpin provide the energy requirement to destabilize hydrogen bonding of the first few bases in the hairpin stem (110).

Expression of *cspA* is regulated posttranscriptionally by a temperature-sensitive RNA switch in its 5 UTR that adopts one of two secondary structures that regulate translation initiation (44). At 37°C, the 5′ UTR forms a secondary structure that sequesters the SD sequence and translation initiation codon in a base-paired structure that inhibits ribosome binding. After a temperature shift to 10°C, the 5′ UTR of *cspA* adopts an entirely different secondary structure in which the SD sequence and AUG start codon are no longer sequestered in a stable structure. This thermosensitive switch controlling ribosome accessibility is the first example of an RNA structure than can promote gene expression at low temperatures (44).

CspA regulates its own translation in an autoregulatory feedback mechanism during cold shock acclimation. As the cellular concentration of CspA increases in response to cold shock, it binds to the 5 UTR of its own mRNA, forcing a conformational rearrangement (167). This rearranged structure in the *cspA* 5′ UTR resembles the structure of the mRNA at 37°C, where translation is blocked and the transcript is rapidly degraded. The structure adopted by the mRNA is dictated by long-distance base-pairing interactions that include a conserved cold-box sequence in the 5′ UTR (42). This base-pairing interaction is a key feature in the structural rearrangement of the mRNA at a temperature shift or in the presence

of bound protein (167). Moreover, limited accessibility of the SD and AUG start codon when the mRNA adopts this structure leads to rapid turnover of the *cspA* transcript (21).

During the acclimation phase of cold shock, the rate of protein synthesis decreases dramatically (67). This decrease is a result of depleted ribosome density in the 5′ ends of open reading frames (ORFs) and increased secondary structure of global mRNAs (167). Recovery of translation is directly correlated with a decrease in ORF mRNA secondary structure mediated by the activity of CspA. A mutational analysis identified RNase R as a second cold shock protein involved in global translational recovery (167). RNase R is required for the turnover of *cspA* mRNA at low temperatures when CspA is bound so as to achieve the appropriate cellular CspA:mRNA ratio (167). RNase R also regulates global mRNA turnover during cold shock acclimation in cooperation with the activity of CspA. Degradation of global mRNAs facilitated by RNase R during cold shock allows the cell to retain the correct CspA:mRNA ratio for efficient RNA remodeling (167).

CsrA-Mediated Translational Regulation

E. coli CsrA and its homologs in other organisms (e.g., CsrA, RsmA, RsmE) have been shown to repress translation of numerous genes. In many instances, translational repression leads to rapid turnover of the mRNA. Translational repression typically involves CsrA binding to multiple sites in the target transcript, one of which overlaps the SD sequence of the mRNA target, such that bound CsrA prevents ribosome binding (112, 143) (Figure 5a). The first such repression mechanism was identified for *E. coli glgC*, which encodes an enzyme involved in glycogen biosynthesis (22). CsrA binds to four sites in the *glgCAP* 5′ UTR, one of which overlaps the *glgC* SD sequence while the other three are located further upstream. CsrA homodimers are capable of bridging a high-affinity site in the *glgC* 5′ UTR to a lower-affinity site overlapping the SD sequence (112). A structural study of a *Pseudomonas fluorescens* RsmE-RNA complex confirmed that protein dimers are capable of binding to two sites within the same transcript (39). Since CsrA-binding sites resemble SD sequences, dual site bridging appears to be a common mechanism to allow effective CsrA-mediated repression while at the same time maintaining a strong SD sequence for translation initiation.

In addition to the general CsrA-mediated translational repression mechanism described above, there are several examples that deviate substantially from this mechanism. CsrA is capable of repressing translation of *hfq* by binding to a single site that overlaps its SD sequence (112). Since Hfq functions as an RNA chaperone that promotes numerous sRNA-mRNA base-pairing interactions (72, 116), the finding that CsrA represses *hfq* expression established a link between these two important global posttranscriptional regulatory systems. Another interesting example was described for the *Pseudomonas aeruginosa psl* transcript, required for biosynthesis of a biofilm matrix polysaccharide of this organism. RsmA binding to the *psl* 5′ UTR stabilizes an RNA structure that sequesters its SD sequence (62). Finally, CsrA represses translation of *iraD*, a gene encoding a protein that prevents targeted degradation of RpoS, the general stationary-phase and stress-response sigma factor of *E. coli*. Rather than directly repressing *iraD* translation, CsrA represses translation of a short leader peptide whose termination codon overlaps the *iraD* initiation codon. Since

translation of the leader peptide and *iraD* is coupled, CsrA represses IraD synthesis entirely via translational coupling (99).

While translation repression is the most-studied mechanism by which CsrA controls gene expression, there are two reported examples of translational activation, although the detailed mechanisms have not been elucidated. *P. aeruginosa* RsmA activates translation of the phenazine biosynthetic gene cluster *phz2*, presumably by destabilizing an SD-sequestering hairpin (109). In addition, *E. coli* CsrA activates translation of *moaA* by altering the structure of the 5′ UTR, thereby promoting ribosome binding (101).

CsrA has also been shown to regulate gene expression via transcription attenuation and mRNA stabilization. The *E. coli pgaABCD* operon encodes proteins involved in the synthesis and secretion of a polysaccharide adhesin involved in biofilm formation (63, 148). In addition to CsrA-mediated repression of *pgaA* translation initiation (148), expression of the *pga* operon is regulated by a Rho-dependent transcription attenuation mechanism (43). In the absence of bound CsrA, an RNA secondary structure forms that prevents Rho from binding to the nascent transcript. However, when CsrA is bound to the *pga* 5′ UTR, this structure cannot form, thereby exposing a Rho-binding site, leading to Rho-dependent termination upstream of the *pgaA* coding sequence. Lastly, *E. coli* CsrA activates expression of the genes encoding a DNA binding activator of flagella biosynthesis and chemotaxis. CsrA binding to the 5′ end of the *flhDC* transcript stabilizes the mRNA by blocking the 5′ end–dependent cleavage pathway of RNase E (153). Since CsrA has been shown to bind hundreds of RNAs in *E. coli* and *Salmonella enterica* (59, 106), it is likely that future studies will reveal new CsrA-dependent regulatory mechanisms.

RNA-BINDING PROTEINS EXHIBITING COMPLEX INTERACTIONS WITH sRNAs

Csr (Rsm) Family Proteins

Competitive inhibition was introduced to the world of sRNAs and RNA-binding proteins when the global regulatory protein CsrA of *E. coli* was found to copurify in a large globular complex with a 369-nucleotide RNA molecule, CsrB, that antagonizes CsrA activity (79, 80, 113). CsrB RNA has 22 GGA-containing sequences, most of which are bound by CsrA (141) (Figure 5b). *E. coli* also expresses a second sRNA, CsrC, related structurally and functionally to CsrB (149). The GGA-containing binding sites of Csr family sRNAs are also closely related to mRNA consensus binding sites derived from SELEX and CLIP-seq studies (16, 36, 59, 106). Except for their repeated GGA-containing stem-loops, the Csr family sRNAs are not highly conserved (39, 64, 114, 143). In a well-studied example, the *P. fluorescens* RsmE:RsmZ complex was shown to be assembled via ordered and cooperative protein binding, although the structural diversity of Csr family sRNAs suggests that this result may not apply to all such interactions (39).

Phylogenetic examinations suggest that the CsrA-inhibitory sRNAs probably function throughout the *Gammaproteobacteria*. Species of this bacterial class are unique in expressing a conserved two-component signal transduction system, referred to as GacS-GacA, BarA-

UvrY, BarA-SirA, etc., that is frequently, but not exclusively, dedicated to the transcription of Csr family sRNAs (27, 57, 165). Candidate CsrA-binding sRNAs have been identified by bioinformatics analyses in a variety of species (41), but to our knowledge no CsrA-sequestering sRNA has been confirmed outside of the *Gammaproteobacteria*.

Because Csr family sRNA levels control CsrA activity and widely influence physiology and virulence gene expression (112, 143), the syntheses of these RNAs have been investigated in several species, most intensively in *E. coli*. CsrB/C transcription responds to cues associated with limitation of nutrients, e.g., ppGpp, the elicitor of the stringent response; carboxylic acid–containing carbon metabolites such as formate, acetate, or TCA cycle intermediates; and the cAMP-Crp complex (28, 31, 40, 97, 123, 165). In addition, the expression of CsrB/C is subject to other conditions and regulatory factors, including quorum sensing (SdiA), envelope stress (σ^E), and DeaD-box RNA helicases (DeaD, SrmB), consistent with a far-reaching role of these sRNAs in monitoring and responding to cell physiology (133, 142, 157). The regulatory interactions between the Csr components themselves and other factors generate negative-feedback loops that support quick and potent responses to changing conditions (1, 97–99, 114, 132, 133, 143, 157) (Figure 5c). Numerous transcription factors are subject to CsrA regulation (106), including transcription factors that directly or indirectly regulate CsrB/C levels (Figure 5c). Such reciprocal regulatory circuitry implies that the Csr system can reinforce transcriptional regulation at the posttranscriptional level for genes that are regulated by both CsrA and an associated transcription factor (40). In addition, the integrated circuitry of systems in which a stress activates CsrB/C production, while CsrA represses one or more regulatory factors responding to that stress, predicts that the Csr system is involved in initiating and propagating stress responses, as well as restoring the basal activity of the stress response system when the consequences of stress have been resolved (e.g., 40, 99).

Turnover of the Csr RNAs in *E. coli*, *Vibrio cholerae*, and most likely other *Enterobacteriaceae*, *Vibrionaceae*, and *Shewanellaceae*, requires the activation of RNase E cleavage by a membrane-associated, nonnucleolytic protein that contains degenerate GGDEF and EAL domains (CsrD or MshH) (77, 132, 147). This role of CsrD allows it to impart global effects on gene expression (104). Because CsrD is activated upon its binding to the unphosphorylated form of $EIIA^{Glc}$ of the PTS pathway, degradation of CsrB/C is triggered by the presence of a preferred carbon source, such as glucose. Limited studies of Csr family sRNAs in species that lack a CsrD homolog find that their decay is directly inhibited by the binding of a Csr family protein (39, 146). In contrast, in *csrD* wild-type *E. coli*, CsrA does not affect CsrB decay (55). However, in strains deleted for *csrD*, CsrB is protected by CsrA against RNase E attack (141). In summary, CsrD is necessary for RNase E to cleave CsrB because this RNA is otherwise protected by CsrA binding, and the evolution of CsrD allowed CsrB/C decay in some species to become responsive to the availability of preferred carbon substrates (77, 141).

The classical Csr family sRNAs, largely composed of repeated GGA-containing stem-loop structures, perform no known function besides CsrA sequestration. However, it seems that other CsrA-binding RNAs sequester CsrA as a moonlighting function, in addition to performing their other roles in the cell (112). These include the abundant mRNA

fimAICDHF of *Salmonella*, to which CsrA binding acts in a hierarchical fashion to prevent CsrA-activated expression of secondary fimbriae (128). The base-pairing sRNAs McaS and GadY seemingly use CsrA sequestration in regulating *pgaABCD* expression and biofilm formation (63, 69, 98, 100, 148). Such regulation is possible because the concentration of CsrA that is not bound to RNA in the cell is low (114), allowing tight-binding RNAs expressed at sufficient levels to compete for CsrA (RsmA) binding with lower-affinity transcripts.

Finally, CsrA-binding proteins have recently been found to serve as CsrA antagonists. One such protein, FliW, participates with CsrA in the regulation of motility in *B. subtilis* and likely functions widely as an inhibitor of CsrA in species outside of the *Gammaproteobacteria* (37, 92, 107, 159). Unlike the Csr family sRNAs, FliW inhibits CsrA activity using an allosteric mechanism (2, 91, 92). On the other hand, CsrA binding to the CesT protein, which also serves as a chaperone for type III secretion system (TTSS) effectors in enteropathogenic and enterohemorrhagic *E. coli* (EPEC and EHEC), inhibits CsrA activity by directly occluding its RNA-binding surfaces (71, 164). While their CsrA-inhibitory mechanisms differ, FliW and CesT both mediate regulation by binding to CsrA after their primary protein partners have been secreted through the pores of the flagellum or TTSS. Notably, EPEC and EHEC strains possess *csrB* and *csrC* genes as well as *cesT*, although their contributions to the regulation of CsrA activity remain to be elucidated (25).

RapZ

The enzyme glucosamine-6-phosphate synthase (GlmS) catalyzes the committed step of the de novo biosynthetic pathway for UDP-*N*-acetyl-D-glucosamine, an essential intermediary metabolite and precursor of peptidoglycan, lipopolysaccharide, enterobacterial common antigen, and the biofilm adhesin poly-β−1,6-*N*-acetylglucosamine (PGA) (24, 63, 70, 111, 127). The widely conserved protein RapZ (RNase adapter protein for sRNA GlmZ) and two sRNAs, GlmZ and GlmY, play a critical role in regulating the expression of *glmS* in *E. coli* (51, 103) (Figure 6a). In its un-cleaved form, GlmZ interacts with the RNA chaperone Hfq, which promotes GlmZ-*glmS* mRNA base-pairing. Bound GlmZ prevents formation of a *glmS* SD–sequestering hairpin, thus activating its translation (70, 140). RapZ binds to GlmZ sRNA and to the N-terminal catalytic domain of RNase E, triggering RNase E cleavage and inactivation of GlmZ, resulting in the loss of translational activation (52). GlmY sRNA is related in sequence and structure to GlmZ but does not bind with high affinity to Hfq or base-pair with *glmS* mRNA (51). RapZ binds to GlmY sRNA. However, unlike GlmZ, GlmY is not cleaved by RNase E upon binding to RapZ; it competes effectively with GlmZ for binding to RapZ (52). This results in stabilization of GlmZ when the active form of GlmY RNA is present. Consequently, conditions that favor GlmY accumulation, such as decreased glucosamine levels, prevent GlmZ cleavage and activate *glmS* translation (52, 108).

Despite their structural similarity, modest differences associated with stem-loop 2 (SL2) (Figure 6b) of these sRNAs permit RNase E to cleave GlmZ:RapZ, but not GlmY:RapZ. The discovery of an RNA aptamer (GlmZ SL1,2) that is recognized by an RNA-binding protein, thus rendering the RNA susceptible to RNase E cleavage, represented a new

mechanism for initiating RNase E cleavage, distinct from the 5′ monophosphate recognition and the direct entry cleavage routes (51). The additional discovery that RNase E cleavage of GlmZ occurs independently of its nucleotide sequence suggested the adaptability of this system for designing regulatable RNA-decay mechanisms, useful in synthetic biology (51). Conceivably, the CsrD protein might invoke a related mechanism for triggering CsrB/C cleavage by RNase E (141), although this remains to be seen.

A crystal structure revealed that RapZ is a homotetrameric protein (48). The N-terminal domain of RapZ is related to kinases, such as adenylate kinase, and contains Walker A and B motifs, while the C-terminal domain is related to 6-phosphofructokinases. While RapZ is unrelated to other RNA-binding proteins, a high density of surface-exposed positive charges in the C terminus suggests this as a likely site for interaction with RNA. These findings have set the stage for the elucidation of the detailed structural biology of this fascinating RNA-binding protein.

ProQ/FinO Family Proteins

ProQ/FinO domain–containing proteins have gained attention as emerging regulatory RNA-binding proteins that possess RNA chaperone activities (58, 95, 125). *E. coli* FinO (fertility inhibition) was once considered a single member of its own class, until recent studies revealed a wider distribution of homologs on the chromosomes or plasmids of *Proteobacteria* (11). Thus far, three different RNA-binding proteins containing the ProQ/FinO domain have been studied (10, 11, 49, 125).

The F plasmid–encoded FinO protein promotes duplex formation between the complementary sequences of the *cis*-acting sRNA FinP and the 5′ UTR of *traJ* mRNA, thus stabilizing FinP against ribonuclease degradation, repressing translation initiation of *traJ* by blocking ribosome binding, and inhibiting plasmid conjugation (10, 45, 65) (Figure 7a). The chromosome-encoded RocC (repressor of competence) protein of *Legionella pneumophila* interacts with the *trans*-acting sRNA RocR to posttranscriptionally repress the expression of competence genes (*comEA*, *comM*, *comEC*, and *comF*) that are required for the DNA uptake by this bacterium (11) (Figure 7b). ProQ itself functions as a monomer and was initially identified as a regulator of the *E. coli* pro-line transporter ProP, although this regulatory mechanism remains to be determined (30). An approach designed to identify the RNA-protein partners in cellular complexes, Grad-seq, suggested the possibility of a global role for ProQ in the posttranscriptional regulatory networks of *Salmonella*, while CLIP-seq enabled the discovery of hundreds of RNA transcripts that directly interact with ProQ in *S. enterica* and *E. coli*. (58, 125). Although most of the currently identified ProQ-binding sRNAs remain uncharacterized, it is clear that only a few (21 out of 138) of its sRNA targets overlap with those of Hfq or CsrA, suggesting the existence of distinct features of ProQ-dependent sRNAs (58). Analysis of the sRNA targets of ProQ revealed that the interaction appears to be driven by recognition of RNA stem-loop structures rather than consensus sequence motifs, which is consistent with observations in FinO- and RocC-dependent sRNAs (11, 58, 65).

One molecular mechanism for ProQ-mediated regulation was identified for the sRNA RaiZ, which interacts at high affinity with ProQ via its two 3′ -terminal stem-loops and the

nucleotides in the 5′ double-stranded region (Figure 7c). RaiZ base-pairs with the 5′ UTR of *hupA* mRNA in a ProQ-dependent manner to prevent synthesis of HU-α by interfering with the formation of a translation initiation complex (126). Notwithstanding its importance as the first mechanistic example of ProQ regulation, this mechanism might not represent the predominant mode of action by ProQ. Indeed, of the ~500 RNAs recognized by ProQ, fewer than 8% were bound at mRNA 5′ UTRs. In contrast, ProQ bound >30% of RNAs at their 3′ ends (58).

An NMR structure of *E. coli* ProQ uncovered an extended architecture with globular N-terminal and C-terminal domains bridged by a flexible linker region (49). All three regions may be involved independently in RNA binding. Notably, the N-terminal domain represents the conserved ProQ/FinO domain, whereas the C-terminal domain not only shares partial similarity with Hfq but also resembles the Tudor domain superfamily of eukaryotic proteins, involved in RNA metabolism and recognition of modified amino acids in protein partners (49, 102). These advances will enable a broader and more detailed molecular understanding of ProQ regulation to be obtained in the future.

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SUMMARY POINTS

1. RNA-binding proteins can regulate transcription termination by attenuation (repression) or antitermination (activation) mechanisms.
2. RNA-binding proteins can regulate translation initiation by altering the accessibility of ribosome-binding sites by promoting or preventing formation of SD-sequestering hairpins and/or by directly occluding ribosome binding.
3. The interactions of RNA-binding proteins with target RNAs can regulate RNA decay by protecting a nuclease cleavage site or by facilitating nuclease recognition of an otherwise inaccessible site.
4. The activity of RNA-binding proteins can be altered by phosphorylation or by binding of effectors including metabolites, sRNAs, or other proteins.
5. RNA-binding proteins can regulate specific genes or function as global regulators by binding to hundreds of RNA targets.
6. RNA-binding proteins can be central to gene expression networks, in which they interact with transcription factors and other regulators to generate diverse and complex biological responses.

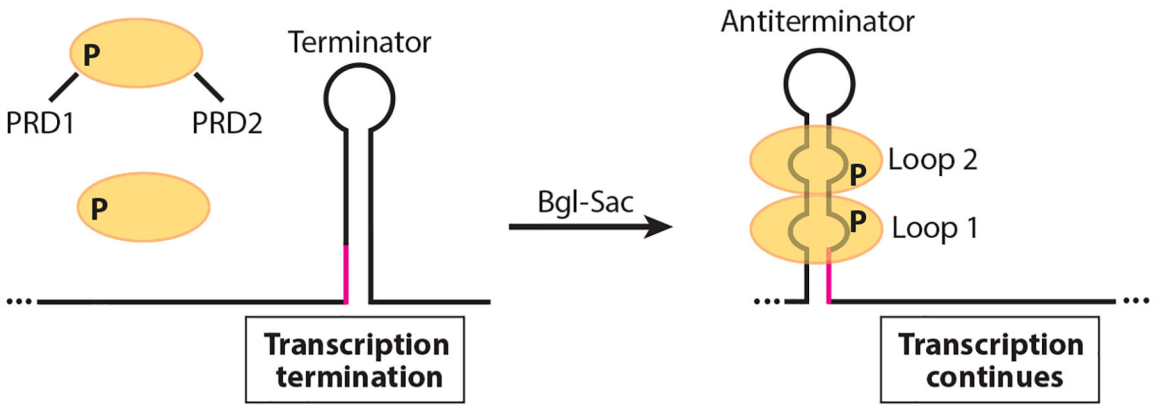

Figure 1.

Model of the Bgl-Sac family antitermination mechanism. In the absence of the cognate carbon source the antitermination protein (*yellow ovals*) is rendered inactive by phosphorylation of PRD1. In the presence of the carbon source, PRD1 is dephosphorylated and PRD2 is phosphorylated, resulting in protein dimerization. The active dimer then binds to its cognate RNA target, thereby stabilizing an otherwise weak antiterminator structure. As a consequence, an overlapping intrinsic terminator is unable to form (overlap in *magenta*), leading to transcription of the operon involved in catabolism of the cognate carbon source. In the absence of bound protein, the terminator hairpin forms and transcription halts upstream of the coding sequence.

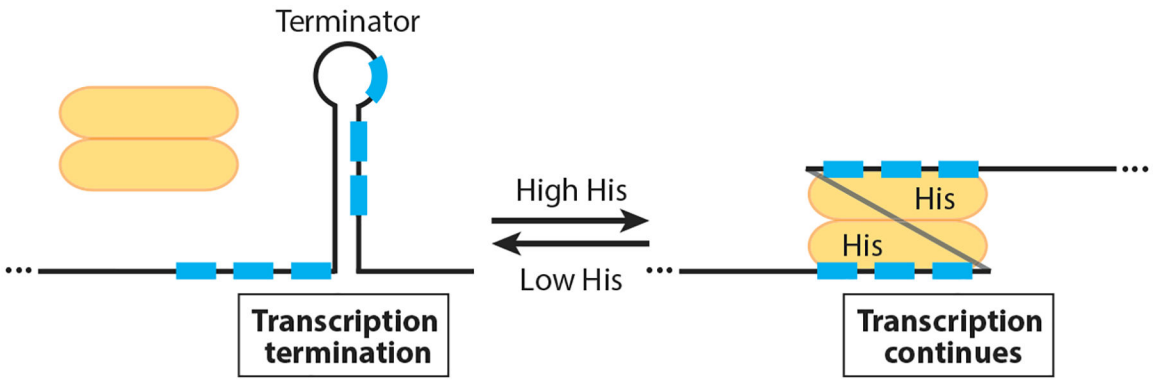

Figure 2.

Model of the HutP antitermination mechanism. Under low histidine conditions, the HutP dimer (*yellow*) is inactive and an intrinsic terminator causes transcription to terminate in the *hutP-hutH* intercistronic region. In the presence of excess histidine, histidine-activated HutP binds to six NAG triplet repeats (*blue boxes*) in the nascent *hut* transcript, thereby preventing formation of the intrinsic terminator such that transcription continues into the downstream histidine utilization genes.

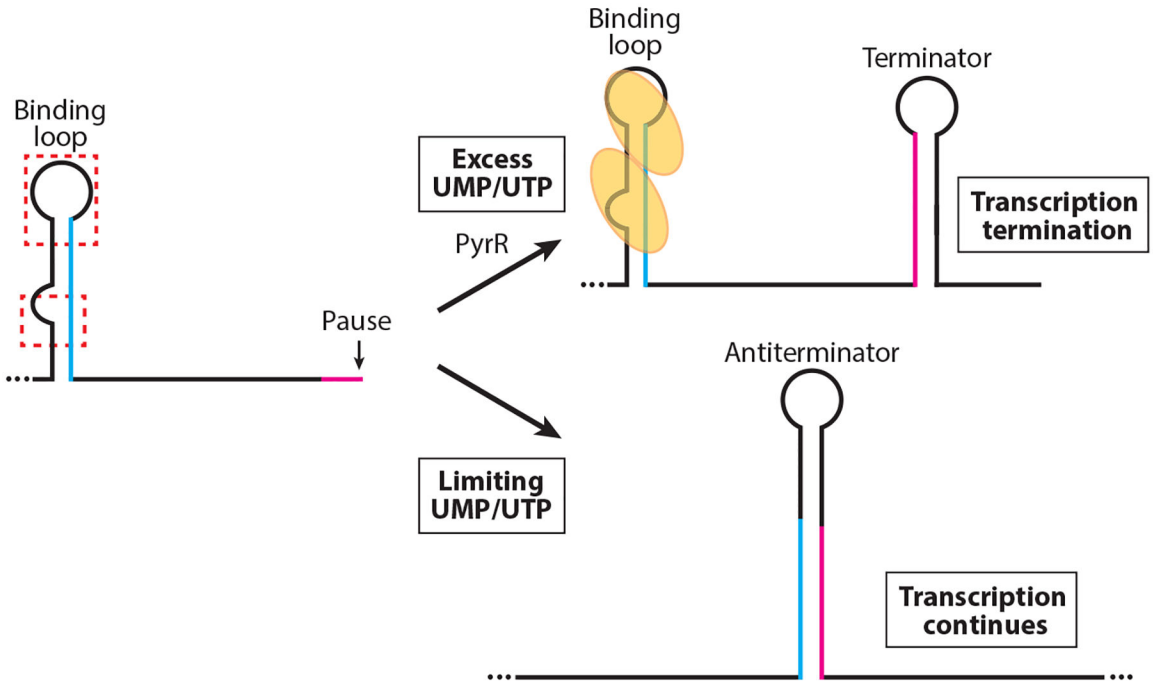

Figure 3.

Model of the PyrR-mediated transcription attenuation mechanism. During transcription RNA polymerase pauses downstream of the PyrR-binding loop and within the overlap between the antiterminator and terminator structures (*magenta*). PyrR (*yellow ovals*) is not activated in limiting UMP/UTP conditions and does not bind to the binding loops in the 5′ UTR, or to the *pyrR-pyrP* and *pyrP-pyrB* intergenic regions. Once RNA polymerase resumes transcription, the antiterminator forms, which prevents formation of the mutually exclusive terminator hairpin (overlap in *blue*). In excess UMP/UTP conditions, UMP/UTP-activated PyrR binds to two regions of the binding loop (*red dashed boxes*). Pausing allows additional time for PyrR binding. Bound PyrR promotes transcription termination by preventing formation of the antiterminator structure.

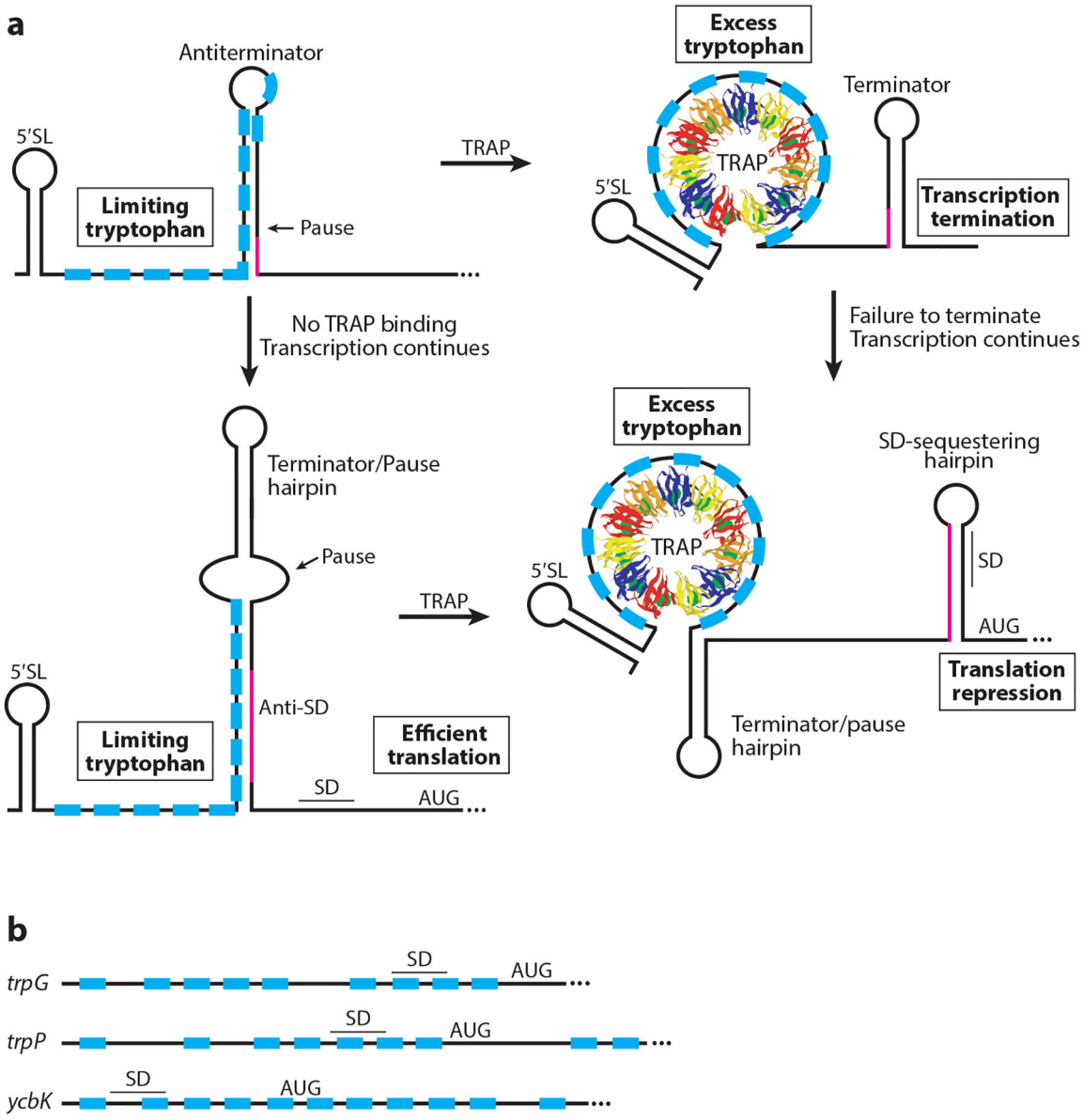

Figure 4.

Models of TRAP-mediated transcription attenuation and translation repression mechanisms. The 11 TRAP subunits are shown in different colors, and tryptophan is shown as green spheres. (*a, Top*) Transcription attenuation mechanism. TRAP is not activated in limiting tryptophan conditions and does not bind to the RNA. Thus formation of the antiterminator results in transcription of the downstream genes. In excess tryptophan conditions, tryptophan-activated TRAP binds to the triplet repeats (*blue boxes*). Pausing may provide additional time for TRAP binding. Bound TRAP prevents formation of the antiterminator, resulting in transcription termination upstream of the coding sequences. Overlap between the antiterminator and terminator structures is shown in magenta. (*a, Bottom*) *trpE* translation control model. In tryptophan-limiting conditions the RNA adopts a structure such that the *trpE* SD sequence is single stranded and available for ribosome

binding. When tryptophan is in excess, tryptophan-activated TRAP binds to its target sequence such that the *trpE* SD–sequestering hairpin forms, causing translational repression. RNA polymerase pausing provides additional time for TRAP binding. Overlap between the two alternative structures is shown in magenta. (*b*) TRAP-binding sites overlapping the *trpG*, *trpP*, and *ycbK* SD sequence and translation initiation regions. Bound TRAP represses translation of each gene. Abbreviations: 5′ SL, 5′ stem-loop; SD, Shine-Dalgarno; TRAP, *trp* RNA–binding attenuation protein. Adapted with permission from Reference 14.

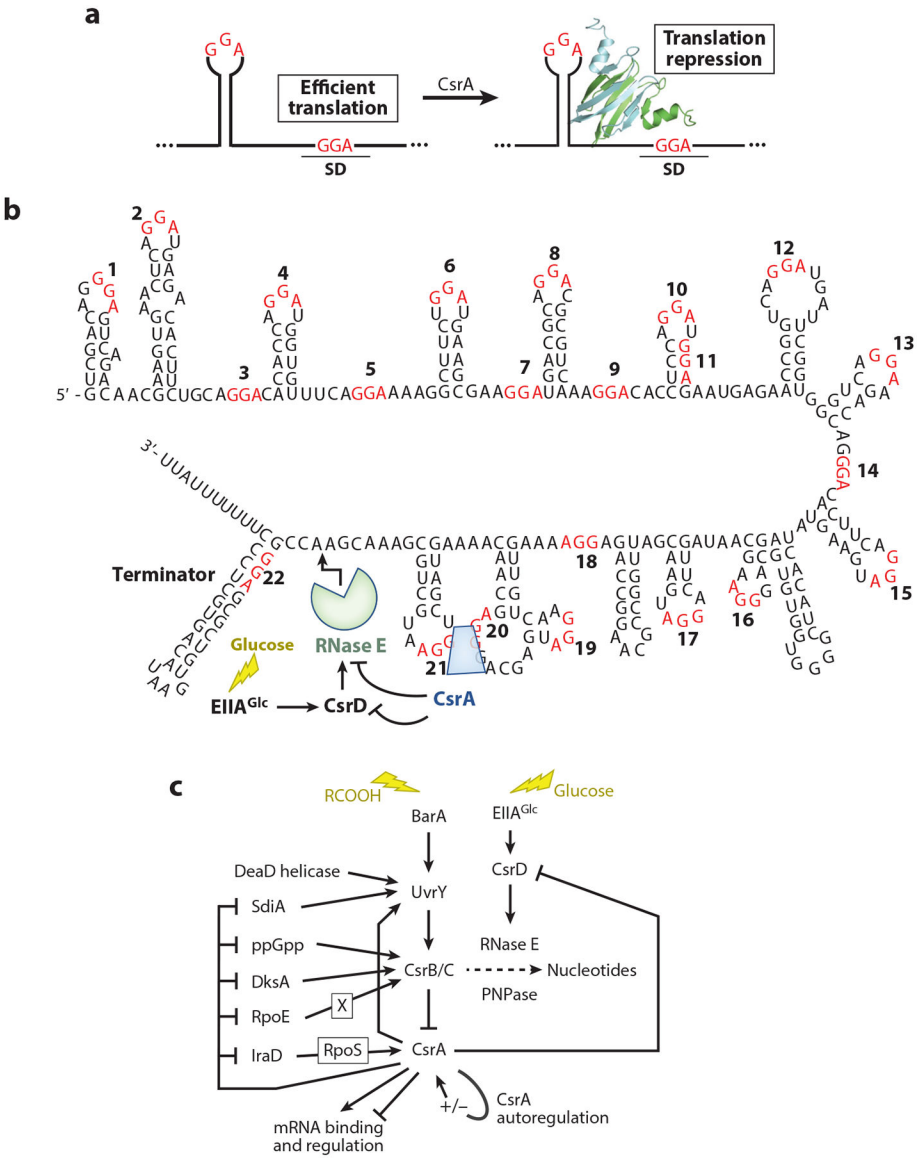

Figure 5.

Csr-mediated regulatory pathways. (*a*) Common CsrA-mediated translation repression mechanism. CsrA dimers, depicted as green and blue ribbons, bind to two sites, one of which overlaps the Shine-Dalgarno (SD) sequence such that bound CsrA blocks ribosome binding. GGA motifs of CsrA-binding sites are in red. (*b*) CsrB sequence, secondary structure, and RNA-decay pathway. GGA motifs are numbered and shown in red. (*c*) Circuitry influencing the Csr system. The Csr system of *Escherichia coli* includes CsrA autoregulation, negative-feedback loops among the Csr components, and reciprocal interactions with other global regulatory systems. Autoregulation and feedback loops fine-tune CsrA activity and support robust regulatory responses, while interactions with other global regulatory systems integrate the Csr system into stress responses. The CsrA ribbon diagram in panel *a* is adapted with permission from Reference 112.

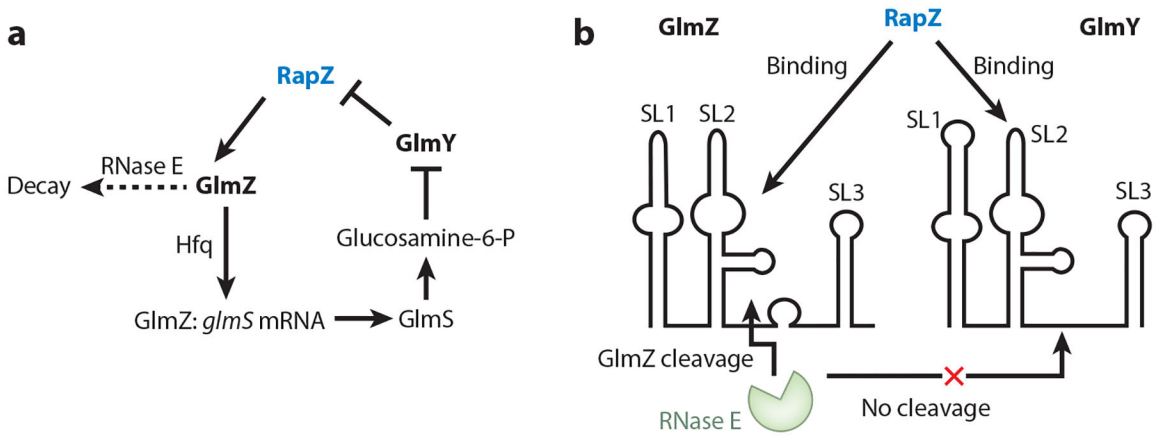

Figure 6.

Regulation by the RNA-binding protein RapZ and its two related small RNA targets, GlmZ and GlmY. (*a*) Regulatory circuitry and (*b*) RapZ:GlmZ recognition involving stem-loop 2 (SL2) promote RNase E recognition and sequence-independent cleavage at the single-stranded base-pairing site of GlmZ. GlmY competes with GlmZ for RapZ binding without undergoing RapZ-mediated RNase E cleavage.

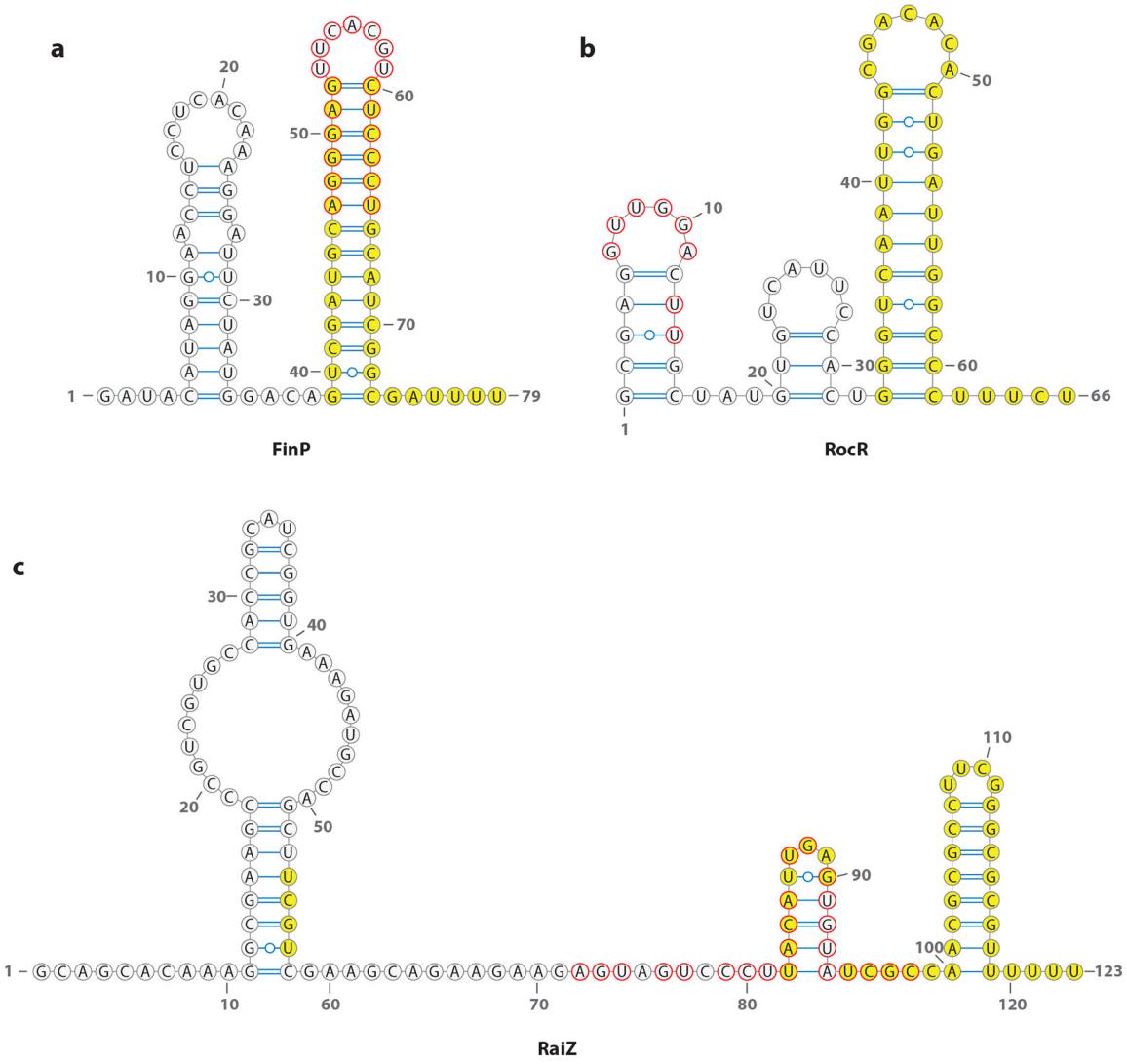

Figure 7.

Target small RNAs (sRNAs) of FinO/ProQ domain proteins. Putative ProQ-binding sites in yellow. Regions involved in base-pairing with target mRNAs are circled in red. Guanine-cytosine pairs are depicted by double bars, adenine-uracil pairs are depicted by single bars, and bars with circles represent guanine-uracil pairs. (*a*) *cis*-Acting FinP sRNA from *Escherichia coli* F plasmid, which is bound by FinO. (*b*) *trans*-Acting RocR sRNA from *Legionella pneumophila*, which is bound by RocC. (*c*) *trans*-Acting RaiZ sRNA from *Salmonella enterica*, which is dependent on ProQ.