



MICROCOMMENTARY

Regulation outside the box: New mechanisms for small RNAs

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Abstract

Regulation at the post-transcriptional level is an important mode of gene expression control in bacteria. Small RNA regulators (sRNAs) that act via intramolecular base-pairing with target mRNAs are key players in this process and most often sequester the target's ribosome binding site (RBS) to down-regulate translation initiation. Over the past few years, several exceptions from this mechanism have been reported, revealing that sRNAs are able to influence translation initiation from a distance. In this issue of *Molecular Microbiology*, Azam and Vanderpool show that repression of the *manY* mRNA by the sRNA SgrS relies on an unconventional mechanism involving a translational enhancer element and ribosomal protein S1. Binding of S1 to an AU-rich sequence within the 5' untranslated region of the *manY* transcript promotes translation of the mRNA, and base-pairing of SgrS to the same site can interfere with this process. Therefore, instead of blocking translation initiation by occluding the *manY* RBS, SgrS reduces ManY synthesis by inhibiting S1-dependent translation activation. These findings increase the base-pairing window for sRNA-mediated gene expression control in bacteria and highlight the role of ribosomal protein S1 for translation initiation.

KEYWORDS

enhancer, Hfq, post-transcriptional regulation, small RNA, translation initiation

1 | INTRODUCTION

Control of de novo transcript synthesis in bacteria usually occurs through the interaction of transcription factors with DNA sequences close to or overlapping with promoter elements. However, transcription control can also occur at a distance employing auxiliary regulatory mechanisms (Xu and Hoover, 2001). In analogy, post-transcriptional gene control through small regulatory RNAs (sRNAs) has long been limited to interactions occurring around the Shine-Dalgarno sequence and start codon of the target transcript; a view that has now been challenged by several reports.

sRNAs are found everywhere on the bacterial chromosome and on plasmids. Despite considerable diversity in size and structure, the majority of studied sRNAs control gene expression by a

common mechanism involving the interaction with an RNA chaperone, e.g., Hfq and/or ProQ, and base-pairing with *trans*-encoded target mRNAs (Hör *et al.*, 2018). sRNAs can activate and repress target mRNAs, and a single sRNA may perform both of these functions (Papenfort and Vanderpool, 2015; Kavita *et al.*, 2018). The base-pairing sequences of activated targets are typically located upstream of translation control elements, whereas target repression most often occurs at the ribosome binding site. Studies focusing on the regulation of *ompN* by the RybB sRNA revealed a five codon window downstream of the start codon suitable for translation repression, which overlaps with the sequence space occluded by the 30S ribosomal subunit during translation initiation (Bouvier *et al.*, 2008). While this mode of action may well account for a large fraction of the reported sRNA-target mRNA interactions, translation initiation

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control by sRNAs can also occur through alternative mechanisms that are independent of RNA duplex formation at the RBS. While this commentary will only focus on non-canonical mechanisms of gene repression by sRNAs, it should be pointed out that activation of target genes by sRNAs normally occurs through base-pairing events upstream of the RBS (Papanfort and Vanderpool, 2015).

2 | REPRESSION UPSTREAM OF THE RBS

The 5' UTR (untranslated region) of an mRNA is key for translation initiation control and frequently the site for post-transcriptional regulation. For example, riboswitches and RNA thermometers are typically contained in 5' UTRs (Serganov and Nudler, 2013), and so are translation enhancer elements and ribosome standby sites. Translation enhancer elements and ribosome standby sites both promote the translation of their respective mRNAs, however, the underlying molecular mechanisms vary. Standby sites are single-stranded sequences that allow unspecific binding of 30S ribosomes to mRNAs and thereby antagonize the inhibitory effect of otherwise stable RNA structures sequestering the RBS (Unoson and Wagner, 2007). In contrast, translational enhancer sequences seem to promote ribosome liberation during translation initiation (Takahashi *et al.*, 2013). Despite these differences, accumulating evidence suggests that these auxiliary mechanisms of translation initiation require the aid of additional protein factors such as ribosomal protein S1. S1 is an RNA chaperone that unfolds structured mRNAs and, for example, facilitates translation initiation at the ribosome standby sites of the *tisAB* and *lpp* mRNAs (Andreeva *et al.*, 2018; Romilly *et al.*, 2019). However, it also interacts with unstructured, AU-rich enhancer sequences upstream of RBSs and activates translation of the associated mRNAs (Cifuentes-Goches *et al.*, 2019). Work by Azam and Vanderpool (2020) presented in this issue of *Molecular Microbiology* now shows that the SgrS sRNA can meddle with this regulatory mechanism and inhibit translation initiation by interfering with binding of ribosomal protein S1 to an AU-rich enhancer sequence located in the 5' UTR of the *manY* mRNA (Figure 1). Similarly, masking of the *tisAB* ribosome standby site by the IstR1 sRNA inhibits translation of the transcript (Darfeuille *et al.*, 2007), and so does base-pairing of the GcvB sRNA with CA-rich translational enhancers of the *gltI* and *yifK*

mRNAs (Sharma *et al.*, 2007; Yang *et al.*, 2014). A common theme for these cases is therefore the sequestration of translation activator sequences upstream of the RBS by the sRNAs, however, for the *gltI* and *yifK* mRNAs it is currently not clear if RNA chaperones such as ribosomal protein S1 are also required in these processes.

Another RNA chaperone associated with the activity of sRNAs is Hfq. The principle role of Hfq is to act as a molecular matchmaker facilitating RNA duplex formation of sRNAs with their target mRNAs (Woodson *et al.*, 2018). Cellular concentrations of Hfq are limiting and successful use of this resource is achieved by active cycling of RNAs on Hfq (Wagner, 2013). As pointed out above, in the majority of cases the base-pairing site of the sRNA overlaps with the RBS, which inhibits 30S ribosome recruitment. However, rather than blocking translation initiation themselves, sRNAs can also exert their regulatory function by depositing Hfq at the RBS (Figure 1). Three examples for this mechanisms have been reported so far: whereas Spot42 interacts with the *sdhC* mRNA upstream of the start codon (Desnoyers and Masse, 2012), the DicF and SgrS sRNAs form RNA-duplexes with the coding sequences of their targets (Azam and Vanderpool, 2018).

Regulation of gene expression through *cis*-acting elements can also involve the activity of additional coding sequences, such as uORFs (upstream open reading frames). uORFs are typically short and therefore frequently overlooked in standard genome annotations (Orr *et al.*, 2019). In *Escherichia coli*, efficient translation of the *fur* mRNA, encoding a master regulator of iron homeostasis, is linked to a 28 aa uORF called *uof* (upstream of Fur). Fur is a transcriptional repressor of the RyhB sRNA, and, *vice versa*, RyhB prevents *fur* translation (Vecerek *et al.*, 2007). Detailed analyses of the molecular mechanism underlying RyhB-mediated repression of *fur* revealed that RyhB blocks translation initiation of *uof* and thereby also down-regulates Fur synthesis (Figure 1). In analogy, GcvB sRNA inhibits the production of the threonine biosynthesis operon by interfering with translation initiation of the *thrL* leader peptide mRNA and similarly repression of *ilvB* by GcvB might be due to targeting of the cognate *ilvL* leader peptide (Sharma *et al.*, 2011). Various ribosome profiling approaches, e.g., Ribo-RET (retapamulin-enhanced Ribo-seq analysis; [Meydan *et al.*, 2019]), have now exposed several previously unknown uORFs, and it will be interesting to test if and how these are involved in post-transcriptional control mechanisms by sRNAs.

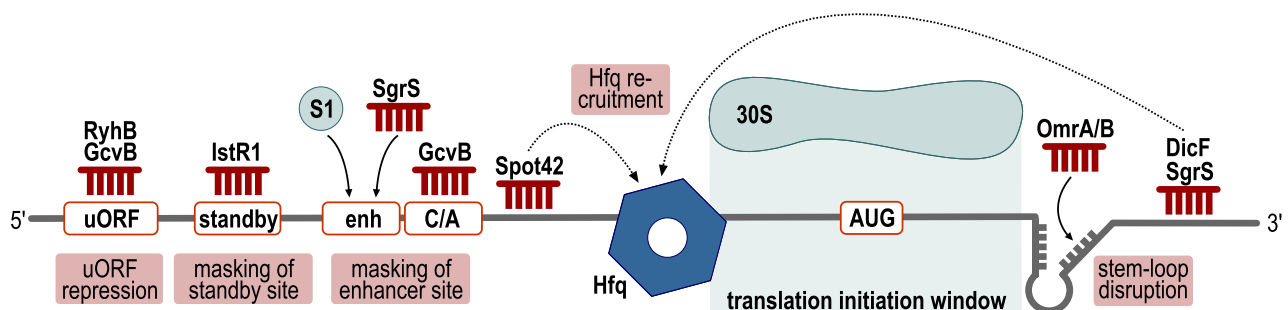


FIGURE 1 Repression of translation initiation through sRNA base-pairing outside the RBS. Bacterial sRNAs employ various regulatory mechanisms to regulate the expression of target outside the window of translation initiation. See main text for details

3 | REGULATION DOWNSTREAM OF THE RBS

Given that elongating ribosomes function as highly processive helicases which should easily be able to unwind sRNA-mRNA hybrids (Takayar *et al.*, 2005), the deeper coding sequence of bacterial transcripts has originally not been considered as a promising base-pairing site. Nevertheless, a number of sRNAs have been identified to regulate expression of their targets through binding downstream of the fifth codon, and thus outside of the region where mRNAs are sensitive to inhibition of translation initiation (Bouvier *et al.*, 2008). Both MicC (Pfeiffer *et al.*, 2009) and SdsR (Fröhlich *et al.*, 2012) do not directly interfere with translation initiation or elongation, and instead act through induction of an RNaseE-dependent cleavage of the *ompD* mRNA immediately downstream of the sRNA binding site, resulting in rapid decay of the transcript. Likewise, down-regulation of *fadL* and *ompA* mRNAs by RybB, and *lpxR* mRNA by MicF (Papenfort *et al.*, 2010; Corcoran *et al.*, 2012), respectively, is based on a similar mechanism.

In contrast to the latter examples, some sRNAs are able to not only destabilize their target mRNAs, but to influence translation initiation from binding sites within the CDS. OmrA and B, two homologous OmpR-controlled sRNAs, act as repressors of numerous mRNAs through occlusion of the RBS (Jagodnik *et al.*, 2017). Instead, base-pairing of the conserved 5' end of OmrA/B within the CDS of the *fepA* transcript interferes with the formation of a short stem-loop within the mRNA. This *cis*-regulatory element increases efficiency of translation initiation, probably by acting as a roadblock to optimize positioning of the 30S subunit. Similar stem-loop structures have been predicted downstream of the AUG in additional mRNAs, suggesting that this mechanism could be more prevalent than previously anticipated. Association of OmrA/B with Hfq increases the stability of the sRNAs, but the RNA chaperone is, at least in vitro, dispensable for repression of *fepA*. Other sRNAs binding within the CDS leverage Hfq to influence translation initiation of their mRNA targets, similar to what is observed for Spot42 base-pairing to the 5' UTR of *sdhC* mRNA (see above and [Desnoyers and Masse, 2012]). Both DicF (encoded by the *E. coli* prophage Qin) and SgrS bind more than 20 nt downstream of the start codon within the *manX* mRNA, the first cistron of the *manXYZ* operon. Association of the Hfq-dependent sRNAs results in deposition of the RNA chaperone more upstream at a site close to the RBS, where it interferes with ribosome binding of *manX* (Azam and Vanderpool, 2018). Based on the idea that the majority of sRNAs recognize their targets within the 5' UTR or the very beginning of the CDS, binding sites further downstream of the RBS have potentially been overlooked in the past. The advent of global approaches dedicated to the unbiased mapping of base-pairing sites, including for example CLASH or RIL-seq (Tree *et al.*, 2014; Melamed *et al.*, 2020), will likely lead to the discovery of more examples of long-distance regulation, and potentially uncover yet unknown mechanisms.

4 | CONCLUDING REMARKS

Interference with translation initiation is the most prevalent mechanism employed by bacterial sRNAs to repress their target transcripts. However, research of the past decade has revealed a repertoire of mechanisms different from the canonical model. sRNAs interacting with sequences up- and downstream of the RBS can influence ribosome assembly from a distance by interfering with the activity of standby or enhancer sites, the regulation of uORF translation, the recruitment of protein factors, or structural rearrangements. Yet, more examples for sRNA-mediated repression through distant base-pairing sites exist, which include the OmrA/B-*csgD*, RybB-*ompC*, RybB-*ompF*, CpxQ-*cfa*, MicV-*ushA*, and ChvR-*chvT* mRNA interactions (Holmqvist *et al.*, 2010; Papenfort *et al.*, 2010; Fröhlich *et al.*, 2018; Bianco *et al.*, 2019; Peschek *et al.*, 2019). The exact regulatory mechanisms for all these latter sRNA-target pairs still remains to be determined and it will be exciting to uncover the ever increasing versatility of bacterial non-coding RNAs within the next years.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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