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Small RNAs in major foodborne pathogens: from novel regulatory activities to future applications

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Small regulatory RNAs (sRNAs) are involved in post-transcriptional control of important cellular processes and contribute to the success of a pathogen. Here, we use studies primarily selected from *Salmonella enterica* and *Listeria monocytogenes* to illustrate the current status of sRNA biology in important foodborne pathogens. We discuss how the regulatory activities of sRNAs can be affected by base pairing RNAs known as 'sponge RNAs', or by RNA-binding proteins, such as the newly discovered sRNA chaperone ProQ. Furthermore, we highlight recent findings for sRNAs with regulatory roles during infection, some of which are present in multiple copies, designated 'sibling sRNAs'. Importantly, knowledge on sRNA-mediated regulation can be exploited for biotechnological applications, such as in generating gene knockdowns to promote desired traits.

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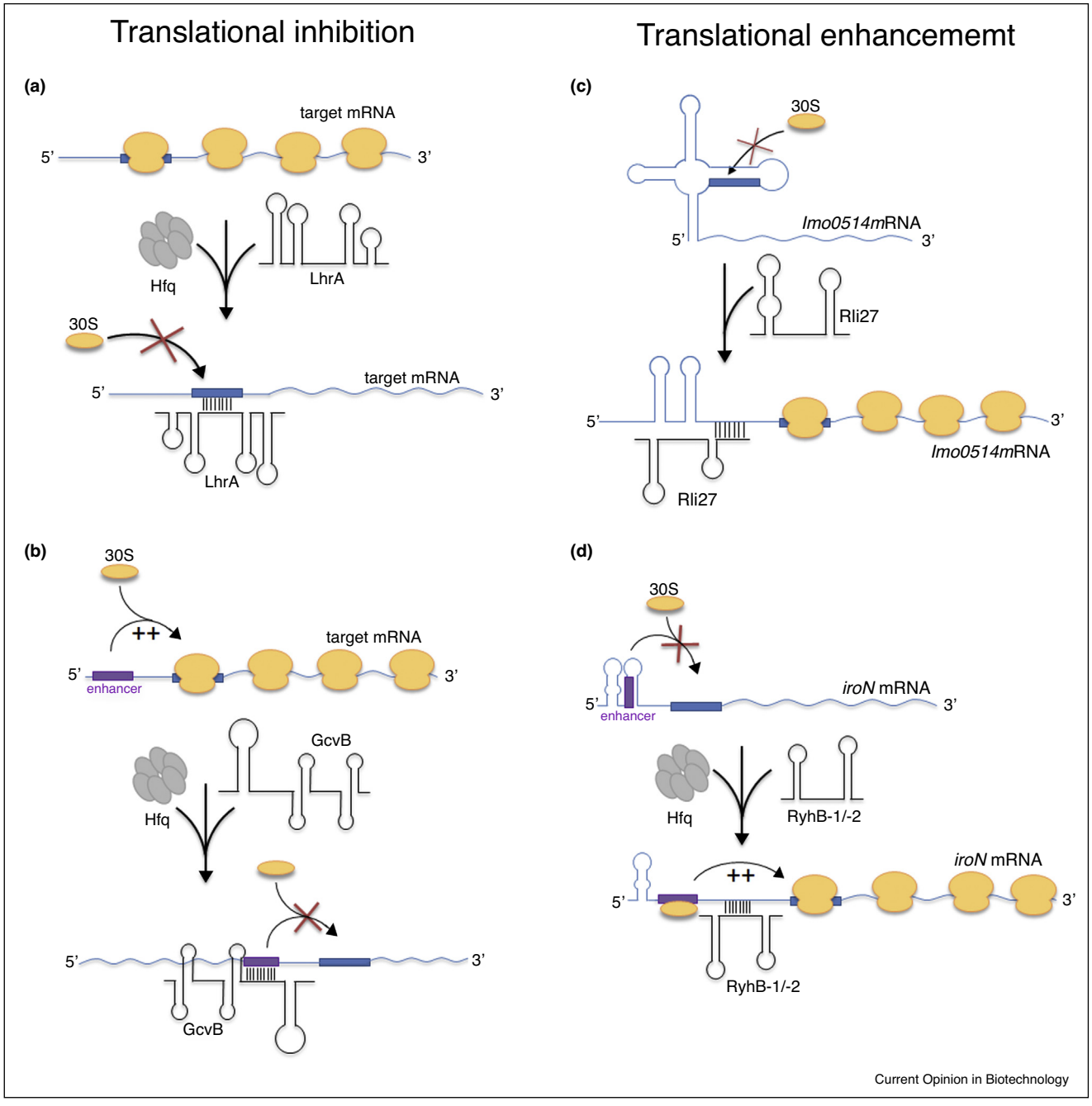
Introduction

Non-coding RNAs serve as regulators of gene expression in bacteria, most often through interactions with other RNA molecules, and influence important cellular processes such as metabolism, stress responses and virulence [1]. In the major foodborne pathogens *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes*, one class of non-coding RNAs, the small regulatory RNAs (sRNAs), has been studied intensively, predominantly in the context of bacterial infection, and several examples of sRNAs that control the expression of virulence genes at the post-transcriptional level are known [2]. Here, we review the latest discoveries in sRNA biology in important foodborne pathogens, with special emphasis on regulatory functions of sRNAs that aid adaptation to host-specific niches. Additionally, we highlight regulatory mechanisms employed by sRNAs, as well as accessory interacting factors, which hold potential for use in synthetic biology and other areas of biotechnology.

Hfq-binding sRNAs — recent discoveries

The largest group of sRNAs in bacteria acts by direct base pairing to specific mRNAs, leading to either inhibition or enhancement of protein expression [1]. *Cis*-acting sRNAs form fully complementary interactions with their target mRNAs, whereas *trans*-acting sRNAs are only partly complementary to their partner mRNAs. In Gram-negative bacteria, the interaction between a *trans*-acting sRNA and its targets often relies on an RNA chaperone, such as Hfq, which promotes sRNA–mRNA duplex formation, whereas in Gram-positive bacteria, a role for Hfq in sRNA-mediated control is less clear [3]. Yet, Hfq contributes to stress tolerance and virulence in both Gram-positive *L. monocytogenes* [4] and Gram-negative *Salmonella* [5]. In early studies, the RNA-binding property of Hfq was successfully used as means to identify sRNAs, such as LhrA in *L. monocytogenes* and GcvB in *Salmonella* [6,7]. Although LhrA and GcvB differ with respect to origin, size and nucleotide sequence, they both rely on Hfq for stability and regulatory activity [8,9]. During growth in rich medium, LhrA accumulates upon entry into stationary growth phase and affects the expression of nearly 300 genes, half of which belong to the regulon of the general stress sigma factor, σ^B [10]. GcvB, on the other hand, is mainly expressed during exponential growth in rich medium and its regulon is highly enriched with genes encoding amino acid-transporters, peptide transporters and amino acid biosynthesis proteins [11]. Both sRNAs use specific seed sequences to pair with complementary sites within the 5′-untranslated region (5′ UTR) of their target mRNAs (Figure 1a and b). Intriguingly, recent findings demonstrate that sRNAs themselves are targets of regulation by other transcripts acting as 'RNA sponges' or 'anti-sRNAs'. One example from *Salmonella* involves a small Hfq-binding RNA, SroC, that derives from processing of the *gltIJKL* mRNA and antagonizes the activity of GcvB by direct base pairing (Figure 2) [12•]. The *gltIJKL* mRNA itself is a target of GcvB, thus, SroC and GcvB together form a feed-forward loop that increases the expression of *gltIJKL*, and moreover, de-represses other targets in the GcvB regulon [12•]. A search for Hfq-binding sRNAs in the enterohemorrhagic

Figure 1



Regulatory mechanisms used by sRNAs — examples of negative and positive effects on translation. **(a)** 'Canonical' sRNA regulation: LhrA in *L. monocytogenes* represses translation of several target mRNAs by base paring in the vicinity of the RBS, thus preventing access of the 30S ribosomal subunit. The Hfq protein stabilizes LhrA and stimulates sRNA–mRNA duplex formation [7,8]. **(b)** GcvB represses translation of multiple mRNAs in *Salmonella* by base pairing to a region upstream of the RBS. Binding of GcvB blocks a CA-rich enhancer site, which acts to stimulate translation [9]. Consequently, base pairing results in translational repression even though the RBS is accessible. **(c)** In intracellular *L. monocytogenes*, the *lmo0514* gene is transcribed with a long 5′ UTR, which forms an inhibitory secondary structure that sequesters the RBS. When the sRNA Rli27 base pairs with the 5′ UTR, the inhibitory structure is relieved, and the RBS is freely accessible to the ribosome [29•]. **(d)** The RyhB siblings in *Salmonella* base pair with the 5′ UTR of *iroN* mRNA, causing a structural change in the mRNA that exposes an enhancer site to which the 30S ribosomal subunit binds [41•]. The stimulating effect of RyhB on *iroN* expression depends on this enhancer sequence, but it is unknown whether 30S binding to the enhancer stabilizes the mRNA or facilitates 30S sliding into the RBS. Blue box, RBS; yellow ovals, ribosome; purple box, enhancer site; base pairing is indicated by thin lines.