Review

New insights into small RNA-dependent translational regulation in prokaryotes

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Bacterial small RNAs (sRNAs) typically repress translation of target mRNAs by pairing directly to the ribosome-binding site (RBS) and competing with initiating ribosomes, an event that is often followed by rapid mRNA decay. In recent years, however, many examples of translation-repressing sRNAs pairing outside the RBS have been described. In this review, we focus on newly characterized mechanisms that explain how a sRNA can modulate translation by binding outside of the RBS and discuss new insights into the events following translation repression. These new mechanisms broaden current perspectives of sRNA pairing sites on mRNA targets and demonstrate how the interplay between sRNAs, mRNA structures, and protein partners can contribute to post-transcriptional regulation.

Small RNAs in bacteria

When they were first characterized in 1984, bacterial sRNAs were the first example of a *trans*-acting regulator controlling translation of specific mRNAs through an antisense mechanism [1]. Since this seminal discovery, sRNA-based regulation has been shown to play major roles in a wide range of organisms, from bacteria to humans. In bacteria such as *Escherichia coli*, where more than 80 sRNAs have been identified [2], sRNAs have been shown to help cells adjust to environmental pressures by modulating the expression of key proteins. Bacterial sRNAs usually base pair with target mRNAs in the vicinity of the RBS to repress their translation and stimulate their rapid decay. By contrast, several examples of sRNAs activating the translation of mRNAs have also been characterized [3–6]. These sRNAs also pair in the 5′-untranslated region (UTR) of their target mRNAs to disrupt inhibitory secondary structures.

In recent years, a large number of studies have uncovered many new putative targets for sRNAs [7–10]. Most of these mRNAs are thought to be regulated by the typical RBS pairing mechanism described above. However, it is now becoming obvious that some sRNAs regulate their targets by alternative mechanisms that involve pairing outside the RBS, therefore excluding a mechanism that relies on direct competition with initiating ribosomes.

It is interesting to note that, in metazoans and plants, a large class of small non-coding RNAs termed miRNAs has also emerged as key players in post-transcriptional gene regulation. Similar to bacterial sRNAs, miRNAs mediate translation repression and mRNA decay. However, unlike their prokaryotic counterparts, they do so by pairing mainly in the 3′-UTR of their target mRNAs. The mechanism of action of miRNAs has been described in recent reviews [11–13].

In this review, we focus mainly on these new mechanisms of action of bacterial sRNAs, with a special emphasis on the recent discoveries that have uncovered new ways by which sRNAs repress translation. We also discuss new insights into the events that follow translation repression. Aside from a few exceptions, this review is limited to work performed on the enterobacteria *E. coli* and *Salmonella*.

Canonical model for sRNA-mediated gene regulation

Translation initiation in bacteria

During protein synthesis, translation initiation is the most rate-limiting and highly regulated step [14]. The canonical model for prokaryotic translation initiation involves mRNA recognition by the 30S subunit of the ribosome, which is mediated by RNA–RNA base-pairing interactions. The 3′-terminal sequence of the 16S rRNA AUCACCUCCUUA (termed antiSD) base-pairs with the purine-rich Shine-Dalgarno (SD) sequence of mRNA [15]. The antiSD–SD base-pairing directs the initiation codon to the P site of the 30S ribosomal subunit. Following the arrival of initiator tRNA, the so-called 30S initiation complex is then stabilized by codon–anticodon interactions. Because formation of the initiation complex is highly dependent on RNA–RNA interactions between mRNA, 16S rRNA, and initiator tRNA, any RNA structure that prevents these interactions will negatively affect the translation initiation rate [16]. Accordingly, bacteria have developed regulatory mechanisms, such as RNA thermosensors [17], riboswitches [18,19], and sRNAs [1], that depend on the occlusion of RNA sequences critical for translation initiation.

Translation repression by sRNAs

Most sRNAs that have been characterized so far pair directly to the SD sequence and/or the initiation codon

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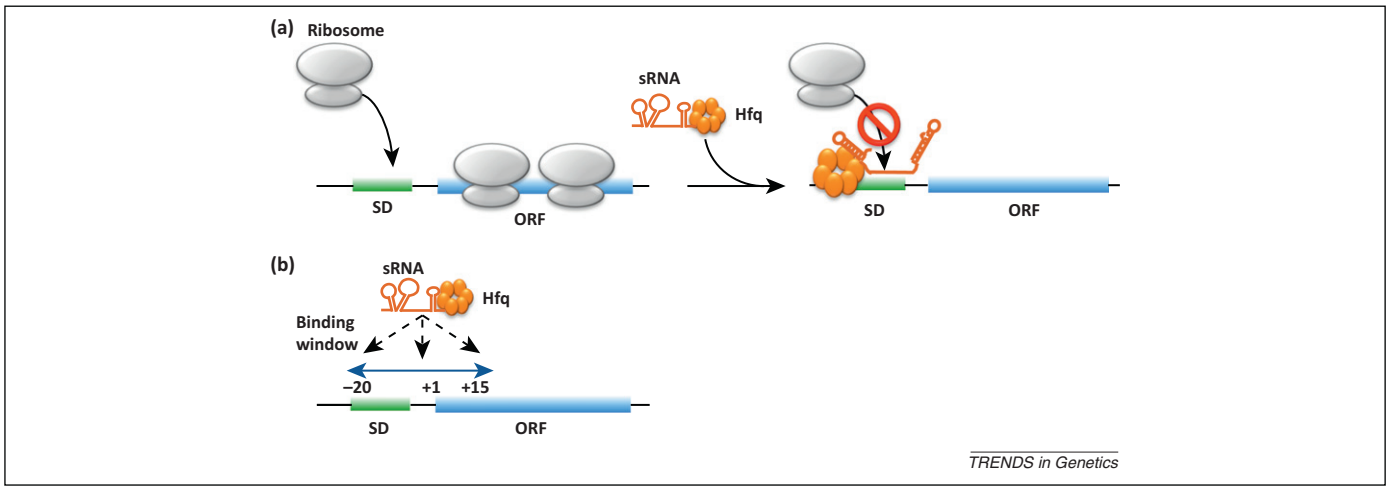

Figure 1. Canonical repression of translation by small RNAs (sRNAs). **(a)** The sRNA, with the help of host factor Qβ (Hfq), blocks translation initiation by directly pairing with the Shine-Dalgarno (SD) sequence in the 5′-untranslated region (UTR) of the target mRNA. **(b)** Binding window of a sRNA blocking translation initiation. The sRNA can pair with a target mRNA anywhere between nucleotides –20 and +15, relative to the first codon. The blue double arrow represents the boundaries of the 30S subunit-binding region. Abbreviation: ORF, open reading frame.

of their target mRNA to repress translation (Figure 1a). This pairing shields translation initiation signals and prevents the binding of the initiating 30S ribosomal subunit. Recently, a 'five codon window' in which sRNAs can efficiently inhibit translation initiation has been identified, meaning that pairing in the upstream part of the open reading frame (ORF) can efficiently inhibit the 30S ribosomal subunit binding to mRNA [20]. This finding is consistent with the previously made observations that the physical boundaries of the RBS extend from nucleotides –20 to +15 relative to the first nucleotide of the start codon (Figure 1b) [21,22]. Although the possibility has not been addressed specifically, it is believed that a sRNA bound up to 20 nucleotides upstream of the initiation codon could also repress translation initiation.

sRNA-induced mRNA degradation

In most cases, translational repression of a target mRNA by a sRNA results in active and rapid mRNA degradation [23–26], a process that is reminiscent of what is observed in eukaryotic cells for certain miRNA-mediated gene repression [27]. In *E. coli*, this degradation is achieved through the recruitment of the single strand-specific endoribonuclease E (RNase E). An additional player involved is host factor Qβ (Hfq) (Box 1), which is an RNA chaperone that has been described to stabilize sRNAs *in vivo* [28,29] and to facilitate pairing to mRNA targets *in vitro* [29–31]. Thus, it is generally thought that Hfq is essential for the activity of many sRNAs. Importantly, it has been shown that Hfq is able to interact with the unstructured C-terminal region of RNase E, linking sRNA-mediated gene regulation to mRNA degradation [25,26]. Importantly, the C-terminal region of RNase E is also used as a scaffold for the assembly of the RNA degradosome, a machinery dedicated to mRNA degradation in *E. coli* [32]. RNase E is thought to perform the rate-limiting initial cleavage that is followed by rapid and complete mRNA degradation. Even though translation repression and mRNA decay are often observed following sRNA expression, the former is sufficient to achieve gene silencing. Indeed, it was shown a few years ago that sRNAs still repress the translation of target mRNA in the absence of RNase E activity [33]. It is believed that mRNA decay is achieved, following translation silencing, to eliminate translationally inactive mRNA and render repression irreversible.

The fact that many target mRNAs are rapidly degraded following sRNA expression has been extensively used as a tool to identify new sRNA targets. The method consists of monitoring the levels of cellular mRNAs shortly after induction (pulse-expression) of the regulatory sRNA using genomic methods, such as microarray analysis and RNA sequencing [7,8,10,34,35]. Because they are observed only a few minutes after sRNA expression (generally <10 min), these changes in mRNA levels are believed to be the result of the direct action of the sRNA. Interestingly, this type of approach also permits the identification of positive mRNA targets, whose activated translation is often associated with increased mRNA stability and levels [3,6].

Box 1. RNA chaperone Hfq

The Hfq protein was initially characterized as a host factor necessary for the replication of the bacteriophage Qβ [63], thus its name Hfq (host factor Qβ). Hfq forms a hexameric ring comprising six identical units of 11.2 kDa. Hfq is a member of the Sm protein family [64], members of which are also found in eukaryotes and Archaea, where they play key roles in mRNA splicing and decay [65].

In prokaryotes, Hfq has been shown to play crucial roles in the action of a large class of bacterial sRNAs [66]. First, Hfq was shown to stabilize sRNAs presumably by protecting them against the action of RNases [28,29]. Hfq is also thought to act as an RNA chaperone that facilitates sRNA–mRNA interactions [29–31,67]. Additionally, Hfq has been directly implicated in translation repression of an mRNA by competing with initiating ribosomes when recruited to the RBS region by a sRNA [48]. Hfq is also implicated in sRNA-induced mRNA degradation. Because it interacts with both the sRNA and RNase E, Hfq is believed to recruit the degradation machinery to the targeted mRNA [23,25,26].

Independently of its role in sRNA-mediated gene regulation, Hfq is also involved in polyadenylation-dependent mRNA decay [68–70], Rho-dependent transcription termination [71], and, finally, transposition [72]. Lastly, Hfq has been shown to repress translation initiation of its own mRNA [73].