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Mini Review

An Overview on the Roles of Bacterial Small RNAs in Regulatory Networks

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Abstract

Bacterial small RNAs (sRNAs) are important molecules that regulate the expression of certain genes, depending on the different growth conditions of the cell and they are widely used by bacteria. sRNAs help the bacteria survive by being involved in many cellular processes such as nutrient deficiency, mobility, pH adaptation and oxidative stress. Current studies have succeeded in elucidating how sRNAs modulate the expression of different transcription factors. Thus, the integration of sRNA activity into comprehensive regulatory networks has begun to take place. Regulatory networks include regulatory circuits that have characteristic functions. In this review, the roles of some characterized sRNAs in regulatory networks and their effects on transcription factors are discussed. Furthermore, we describe specific regulatory circuits containing base pairing sRNAs and their importance in global regulation.

Keywords: Small RNA; regulatory network; transcriptional regulator

Introduction

Microorganisms have to adapt extremely quickly to various challenges, particularly environmental stress conditions and the host immune system. The adaptation of bacteria to these different environments requires constant and strict regulation of gene expression [1]. Studies on bacterial small RNAs (sRNAs) have revealed that these molecules are important regulators of various cellular networks. Besides, they have key roles in mediating responses to environmental stress, regulating virulence and host-pathogen interactions [2,3]. Although it's still a newly studied area, the regulatory roles of bacterial sRNAs have greatly expanded our knowledge of various cellular networks.

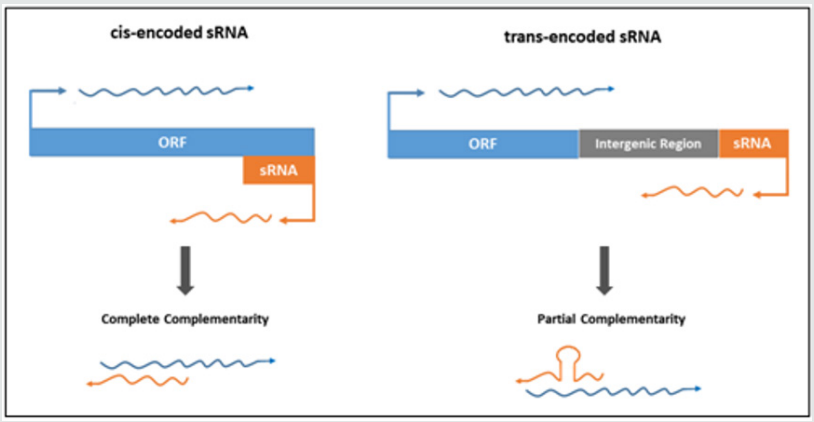

Figure 1: Difference between the mechanism of action of cis- and trans-encoded sRNAs depending on their genomic location. Orange boxes show the sRNA while blue boxes show the target mRNA. Grey box illustrates intergenic region [8].

Bacterial sRNAs are approximately 50-400 nucleotides in length and help to modulate changes in cellular metabolism, especially under stress conditions. As a result, the use of existing nutrients is optimized and the possibility of bacteria survival increases [4]. While some of these regulatory RNAs can modulate the activities of proteins by binding to them, most of the characterized sRNAs act by performing base pairing with target mRNAs [5,6]. sRNAs acting through base pairing are studied in two categories: (i) trans-encoded and (ii) cis-encoded sRNAs see (Figure 1). Trans-encoded sRNAs are encoded at genomic locations that are far from the mRNAs they regulate and therefore they often share limited complementarity with their targets [7].

Difference between the mechanism of action of cis- and trans-encoded sRNAs depending on their genomic location. Orange boxes show the sRNA while blue boxes show the target mRNA. Grey box illustrates intergenic region [8].

Most trans-encoded sRNAs have multiple mRNA targets. In some bacteria, base pairing between sRNAs and their targets require the Hfq protein which is an RNA chaperone. Hfq is a protein commonly found in bacteria that can bind to RNA, which has key roles in controlling gene expression. Hfq affects the translation of specific transcripts by making it easier for sRNAs to match their target mRNAs. Unlike trans-encoded sRNAs, cis-encoded sRNAs are transcribed on bacterial chromosomes from the opposite strand of target genes and therefore they show great complementarity with their targets [9,10]. In recent years, increasing characterization studies of sRNAs have required the participation of these sRNAs in global regulatory networks. In this review, we summarized the various regulatory networks in which sRNAs are located and the roles of sRNAs involved in the process. We are also focusing on the effects of sRNA molecules interacting with mRNA targets.

Regulatory Networks Involving sRNAs

There are numerous transcriptional regulator mRNAs known to be regulated by sRNAs. Therefore, the regulatory networks of sRNAs may be larger than estimated. sRNAs are known to regulate important regulatory networks such as amino acid biosynthesis, quorum sensing system and biofilm formation. This regulation occurs when sRNAs target mRNAs that play a role in regulatory networks. *rpoS* [11], encoding the sigma factor; *csgD* [12] that regulates the synthesis of curli and cellulose; *luxR* and *aphA* [13], the quorum sensing (QS) regulators and *lrp* [14] involved in amino acid biosynthesis can be given as examples to important mRNAs that are regulated by sRNAs.

In enteric bacteria, RpoS (RNA polymerase sigma factor) protein, a global regulator, is required to adapt to different stress conditions. Stress stimuli, including stationary phase, nutrient deficiency, low temperature, and osmotic shock, all cause a significant increase in RpoS production and activity [15]. In addition, these different stress conditions can lead to activation of Hfq-dependent specific sRNAs; DsrA, RprA and ArcZ, which stimulate RpoS translation. In the absence of these three Hfq-dependent sRNAs, there is a secondary structure that hides the ribosome binding site (RBS) in the 5′ UTR of the rpoS mRNA. Each sRNA interacts with this secondary structure of the rpoS mRNA through a similar mechanism. Then, they open the hairpin structure and make the ribosome binding site accessible. Thus, translational inhibition of *rpoS* mRNA is eliminated and the translation is enhanced [16]. OxyS is a sRNA with a length of about 110 nucleotides that negatively regulates the translation of *rpoS* mRNA. Under the oxidative stress conditions, OxyS is thought to suppress the translation of *rpoS* mRNA by sequestering Hfq instead of binding to *rpoS* mRNA [17].

CsgD, the main regulator of biofilm formation, is a transcription factor that activates the synthesis of curli fimbria and extracellular polysaccharides in *Escherichia coli* and *Salmonella* Typhimurium. CsgD acts as a transition between planktonic and biofilm life forms by coordinating the expression of genes involved in mobility and adhesion [18]. The sRNAs (DsrA, ArcZ and RprA) mentioned earlier are modulates RpoS expression and also indirectly control the production of main biofilm matrix components, curli fimbriae and cellulose. In addition, sRNAs called RprA, McaS, OmrA, OmrB, GcvB, RybB and RydC cause negative regulation of the *csgD* mRNA by base pairing with the 5′ UTR. Therefore, they also suppress biofilm formation [19].

GcvB is one of the small RNAs associated with Hfq, which is highly conserved in Gram-negative bacteria. It provides post-transcriptional control of genes involved in amino acid metabolism and acid stress. GcvB is known to directly inhibit the expression of the transcription factor Lrp, which plays a role in amino acid biosynthesis. Also, Lrp and GcvB are thought to function together in a mutually inhibiting regulatory circuit for controlled regulation of cellular amino acid availability [20].

The system where bacteria communicate with each other using extracellular signal molecules known as autoinducer is called quorum sensing. Bacteria regulate this communication process by secreting small signaling molecules out of the cell and sensing molecules secreted by other bacteria [21]. Five homologous sRNAs that can regulate the QS system have been discovered in *Vibrio harveyi* and *Vibrio cholerae* [22]. These sRNAs have been called quorum regulatory small RNAs (qrr sRNAs). The first identified targets of qrr sRNAs are transcription factors, the main regulator of the QS mechanism. These are mRNA transcripts known as hapR in *V. cholerae* and luxR in *V. harveyi*. Ongoing studies have shown that qrr sRNAs quickly modulate QS activities depending on cell density [13,23].

Each of the mentioned sRNAs can not only regulate these transcription factors but also have multiple targets. All these results show the complexity of post-transcriptional gene regulation and the genetic network managed by Hfq and sRNAs. Regulatory circuits involving sRNAs that regulate the *rpoS, csgD, lrp, hapR* and *luxR* mRNAs are shown in (Figure 2).

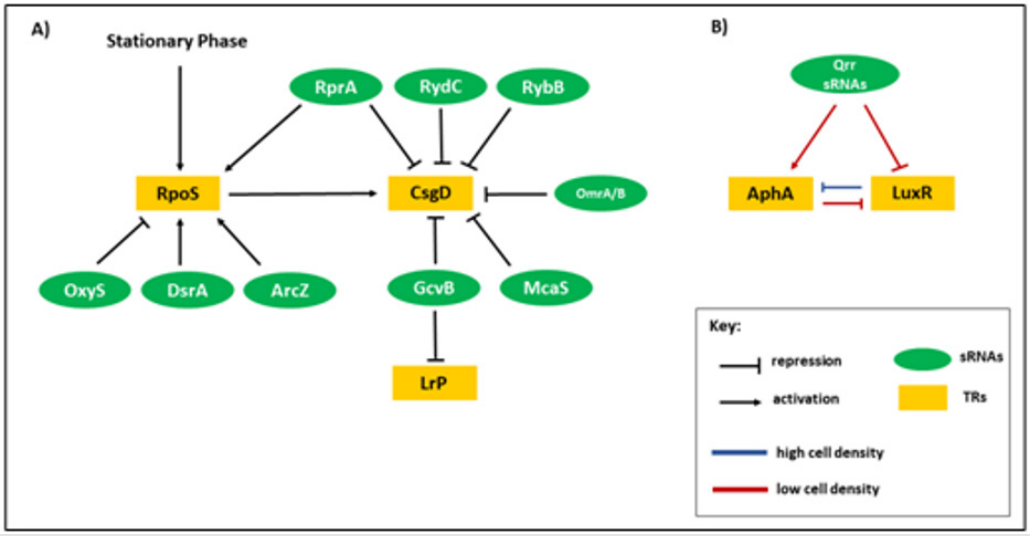

Figure 2: Regulatory circuits involving sRNAs. A) Regulation effects of sRNAs on RpoS, CsgD and Lrp transcriptional regulators (TRs). B) QS regulation based on qrr sRNAs. At low cell density (blue arrows), qrr sRNAs stimulate translation of AphA and repress translation of LuxR. At high cell density (yellow arrow), qrr sRNA production is stopped. Therefore, the translation of AphA is stopped and the translation of LuxR is initiated [24, 25].

At high cell density (yellow arrow), qrr sRNA production is stopped. Therefore, the translation of AphA is stopped and the translation of LuxR is initiated [24,25].

Conclusion

Bacterial sRNAs are important regulators that provide control of gene expression under specific growth conditions. These RNA molecules, via regulating gene expression, can help bacteria adapt to new environmental conditions and respond to various stresses. In such a situation, sRNAs rapidly control the expression of target genes, increasing the chances of bacteria surviving. sRNAs can modulate transcription, translation, and mRNA stability both positively and negatively. sRNAs that act by base pairing directly regulate gene expression by interacting with the target mRNAs. This interaction can occur in untranslated region or coding sequence to induce or repress translation of the target mRNA. Also, in many cases, the RNA chaperone Hfq facilitates the interaction between sRNAs and their targets. Moreover, unlike the mentioned mechanisms, some sRNAs can bind to protein targets and sequester their functions.

sRNAs generally have multiple targets, and ongoing characterization studies are beginning to reveal how sRNAs are involved in global regulatory networks. Regulatory networks consist of regulatory circuits that have characteristic behavior and functions. Many transcriptional regulators are known to be regulated by sRNAs. The sRNA-mediated regulation of these transcriptional regulators led to the construction of regulatory networks.

Thus, here, we review examples of sRNAs that are involved in numerous cellular processes, such as stress response, adaptation to growth conditions, quorum sensing (QS) and biofilm formation. Research on bacterial sRNAs has many unanswered questions. However, as a result of increased studies in which characterizations of sRNAs are performed, new regulatory circuits will emerge. Then we will have a better understanding of how sRNAs are integrated into regulatory networks and why they exist.

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