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Review article

Non-Coding RNAs in Bacteria

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Abstract

Genes encoding regulatory RNAs known as short *RNAs* (sRNAs) or *non-coding sRNAs* (ncRNAs), modulate physiological responses through different mechanisms, through RNA-RNA interaction or RNA-protein interaction. These molecules transcribed *in trans* and *in cis* relative to the target RNA. They are located between the coding regions of proteins, i.e., in the intergenic regions of the genome and show signals of promoters and termini sequences generally Rho-independent. The size of the ncRNAs genes ranges from ~ 50 to ~ 500 nucleotides and several transcripts are processed by RNase with smaller end products, which modulate physiological responses through different mechanisms, by RNA-RNA interaction or RNA-protein interactions and some interactions may be stabilized by the Hfq chaperone. The *Riboswitches* constitute another class of ncRNAs, located in the 5'UTR region of an mRNA that promote transcriptional regulation through their interaction with a linker molecule. Recently, in prokaryotes, CRISPR (*Clustered Regularly Interspaced Short Palindromic Repeats*) regions have described, which repeats of sequences of palindromic bases are. Each replicate consists of short segments of "spacer DNA" from exposures prior to a bacteriophage virus or exogenous plasmid. The CRISPR system consists of an immune system of resistance to exogenous molecules.

Keywords: ncRNA, *cis-encoded* ncRNA, *trans-encoded* ncRNA, *riboswitch*, CRISPR.

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Introduction

Non-coding RNAs can be classified as short non-messenger RNAs (snmRNAs), small non-coding RNAs (ncRNAs), untranslated RNAs (untranslated RNAs) RNAs), or non-protein-encoding RNAs (npcRNAs) [1]. Short RNAs (small RNAs or sRNAs or non-coding RNAs or ncRNAs are molecules that modulate physiological responses through different mechanisms through RNA-RNA interaction or RNA-protein interaction). Some interactions can be stabilized by the chaperone Hfq [2-4], which occurs commonly in the class of trans-encoded ncRNAs where the formation of the ncRNA:Hfq:mRNA complex may act positively or negatively on post-transcriptional regulation [5].

The most studied ncRNAs are the cis-encoded RNAs and the trans-encoded RNAs, the first transcripts being in cis and antisense relative to the target mRNA and the second transcripts in genomic regions distant from the target mRNA [6]. Therefore, only the cis-encoded RNAs present perfect base pairing with the targets. The Riboswitches constitute another class of ncRNAs, located in the 5'UTR region of an mRNA, which promote transcriptional regulation through its interaction with a linker molecule [7-9]. Recently, in prokaryotes, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) regions that are repeats of sequences of palindromic bases have described [6, 10]. Each replicate consists of short segments of "spacer DNA" from exposures prior to a bacteriophage virus or exogenous plasmid. The CRISPR \ cas system consists of an immune system of resistance to exogenous molecules [11].

Computational tools are widely used for the prediction of ncRNAs, such as the QRNA [12] that performs comparative analysis of genomes, the ISI [13] which analyzes conserved intergenic sequences between genomes related to the identification of ncRNAs. The RNAz 2.0 computational program [14] analyzes the thermodynamic stability of the conserved RNA structure and the existence of a promoter and terminator in the possible predicted ncRNAs. The prediction tools sRNAPredict2 [15] and SIPHT [16] evaluate information from the Rho-independent terminator promoters against the database. In addition, the large-scale experimental approach involving cDNA sequencing (RNA-seq) has been widely used in the prediction of ncRNAs and is a very efficient method due to the possibility of confirming the expression of predicted ncRNAs [17-20]. The aim is to contribute to the understanding of the participation of this type of RNA in the regulation of bacterial metabolism.

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2. RNAs NOT ENCODING IN PROCARIOTS

The scientific and technological advance provided the discovery of the functions of those considered as main RNAs: messenger RNA (mRNA), molecule of information transfer during protein synthesis; RNA ribosomal (rRNA) component of the structure of protein synthesis and RNA transporter (tRNA) capable of transporting amino acids and interacting with proteins [21]). Several structural and functional studies of RNA have performed and allowed the description of new classes of RNA, with varied functions and in the *Archea*, *Bacteria* and *Eukaria* domains (Table1) [22].

While genomics considers and analyzes genomes as coding sequences for mRNAs, rRNAs and tRNAs, RNomica further investigates the genes encoding RNAs that are untranslated but are functional RNAs and are involved in different cellular processes [23, 24]. Non-coding RNAs are involved in several cellular processes, such as chromosome replication in cell division (diF), transcription regulation (6S RNA), RNA processing (mRNA), mRNA stability and translation (antisense sequence - spot42), protein stability (tmRNA) and transport (4.5S or ffs) [25]. There are ncRNAs involved in oxidative stress (oxyS), stationary phase transcription (dsrA, rprA), related to the control of plasmid copy number (RNAI, RNAIII), carbon storage (csrBC) and carbon transport (gcvB) [24].

The class of ncRNAs with regulatory function is involved in several regulatory mechanisms such as gene expression in the modulation of outer membrane surface proteins (Omp) [26]. Some ncRNAs may bind to proteins in order to modulate their activities, as is the case with the 6S RNA that forms a complex with RNA polymerase (RNAP) [2, 24, 27]. These molecules are transcribed *in trans* or *in cis* relative to target RNA [4, 28]. The genes are located between the protein-coding regions, that is, in the intergenic regions of the genome and exhibit signals of promoters and terminator sequences, generally Rho-independent [29-32].

The size of the ncRNAs genes ranges from ~ 50 to ~ 500 nucleotides and several transcripts are processed by RNase with smaller end products (Figure 1) [20, 33, 34, 35]. The antisense pairing between the regulatory ncRNA and the target messenger RNA is the most common mechanism of action [36, 37]. This interaction occurs in short regions and imperfect complementarity of sequence, and can be stabilized by the Hfq chaperone protein [26, 38, 39].

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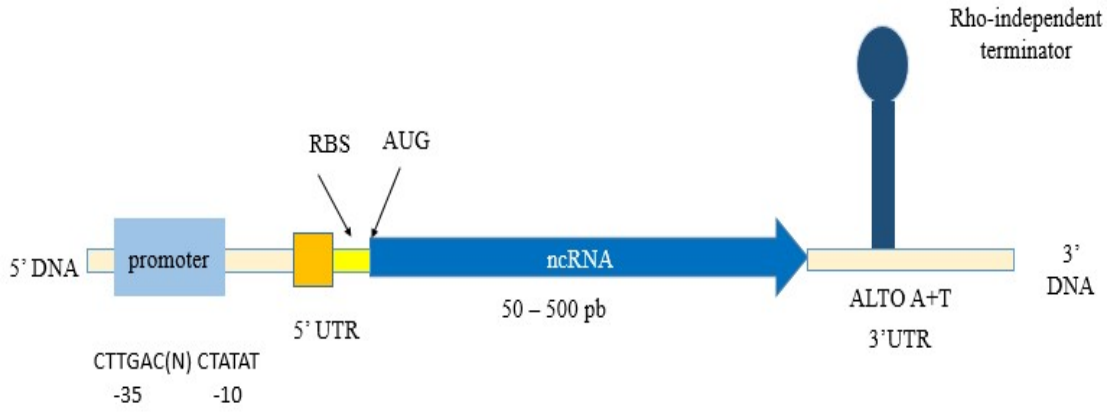

Fig. 1 - Structure of a ncRNAs gene in bacteria, where it has a CTTGAC (N) (-35) and CTATAT (-10) promoter region, ribosome binding site (RBS), an AUG coding region at the 5'UTR. It can vary in size from 50 to 500 base pairs, and in the 3'UTR region it has a terminator sequence (Rho-independent).

Jager and collaborators (2012) [40] demonstrated that the interaction of ncRNA162/ target RNA in *Archaea* can occur with cis-encoded or trans-encoded action, through two distinct domains. As in Bacteria, ncRNAs in *Archaea* are involved in many biological processes, such as metabolic regulation, adaptation to environmental conditions, stress response, regulation of morphology, and cellular behavior. In *Methanosarcina mazei* GO1, Babski *et al*. (2014) [41] identified ncRNAs that align in the 5'UTR region of target mRNAs and in *Sulfolobus solfataricus* ncRNAs that align in the 3'UTR region of target mRNAs. Already for *Pyrobaculum sp*. and *Haloferax volcanii*, there is evidence that ncRNAs can align with target mRNAs at both the 5'UTR end and the 3'UTR end. In addition, they have observed that the nfRNA TRF down-regulates translation in response to binding to RBS.

The mode of action of regulatory ncRNAs depends on co-localization with their target mRNAs (Figure 2). They can be classified into trans-encoded RNA, encoded far from their mRNA targets, and in cis-encoded when located in the 5'UTR region relative to the target. The riboswitches, mostly found in the 5'UTR region, undergo conformational changes in their secondary structure, due to binding of a ligand, and regulate gene expression [27, 34].

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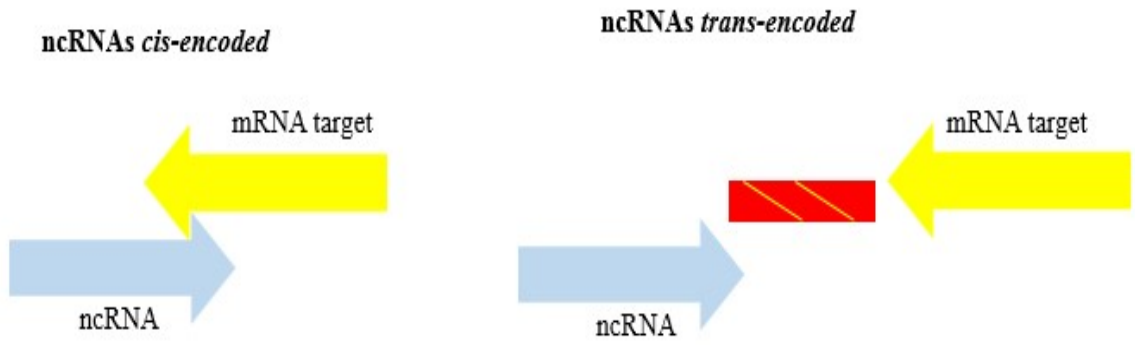

Fig. 2 – Sense of transcription ncRNAs and pairing mode with target RNAs. Genes of *cis-encoded* ncRNAs are located close to the target mRNA encoding genes while *trans-encoded* ncRNAs genes are located far from their target mRNAs. After transcription, the *cis-encoded* ncRNAs make short and perfect pairing with their targets and *trans-encoded* ncRNAs make long and imperfect pairing.

The mechanism of action of ncRNAs varies according to their function (Figures 3 and 4). In the post-transcriptional step the *cis-encoded* ncRNAs can cause mRNA degradation (Figure 3a), translation inhibition (Figures 3b), cleavage of the target mRNA (Figures 3c) (5'UTR *Overlaping*) and transcription termination (Figures 2d) [42]. The interaction of the *trans-encoded* ncRNA with target mRNA may result in inhibition of translation (Figures 4a) and degradation of mRNA by the negative interaction of 5'UTR inhibiting the ribosome binding site, or in the degradation paired with RNase (Figures 4b). The ncRNA *trans-encoded* with its target mRNA may form an inhibitory structure that could somehow block the site of the ribosome binding site, thereby impeding translation (Figures 4.c) [42]. Many *cis-encoded* RNAs with antisense orientation can form binary *trans-encoded* complexes with the target mRNA, which demonstrates that this classification is no rigid or decisive [43].

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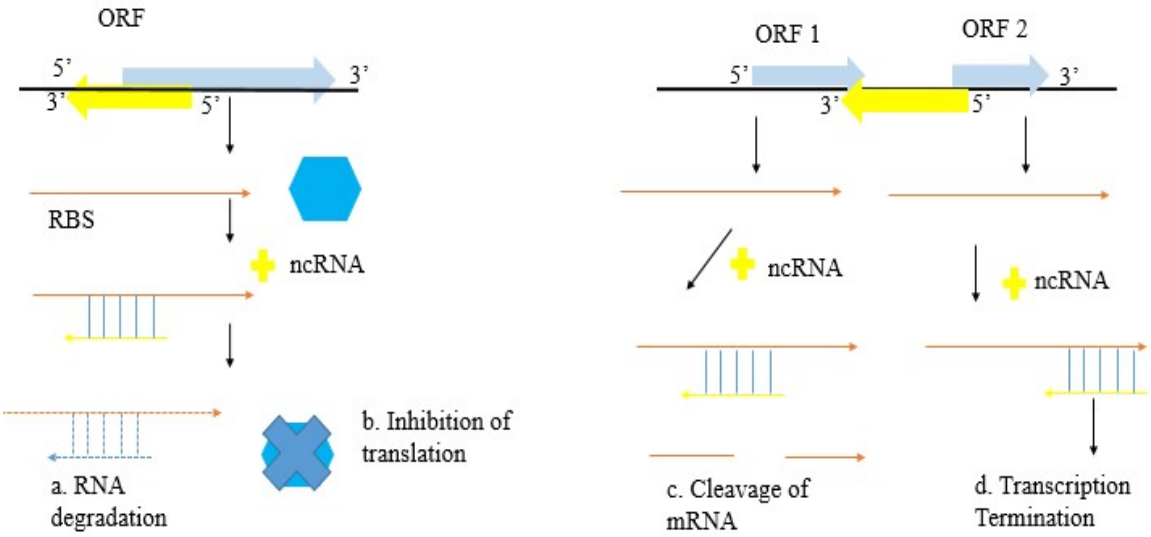

Fig. 3 – Mechanisms of cis-encoded action in which, in the genome, the ncRNA is located close to the gene of its target mRNA. The cis-encoded ncRNAs represented in yellow and their target in blue. 3a. Degradation of mRNA. 3b. Inhibition of translation. 3c. Cleavage of mRNA and 3d. Transcription termination.

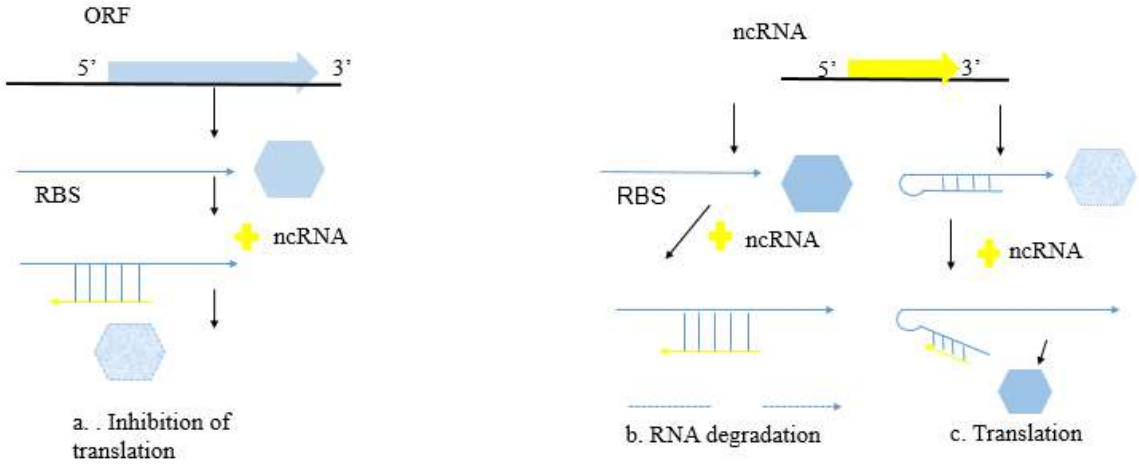

Fig. 4 – Mechanisms of trans-encoded action in which, in the genome, the ncRNA is located far from the gene of its target mRNA. The trans-encoded ncRNAs are represented in red and their target mRNA in blue. Complementarity of base pairs is limited. 3a. Inhibition of translation. 3b. Degradation of mRNA. 3c. Allows translation.

2.1 *cis-encoded* ncRNAs

The *cis-encoded* ncRNAs are present in different bacterial species, Gram-positive and Gram-negative [39, 43]. In E. coli, Wagner and Simons (1994) [44] described the antisense regulatory role of RNA in the control of mRNA gene expression, phage maturation, mobile transposition and plasmid replication. In addition, they may participate in regulating the

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initiation of replication, plasmid conjugation, transposition, mRNA degradation and some cell metabolism pathways. These events continue to be the subject of investigation by different researchers [4, 28, 43, 45].

The *cis-encoded* ncRNAs are encoded at the same locus of their target mRNA, but in the antisense sense of duplex, thus being fully complementary during the interaction. The mechanism of post-transcriptional response of gene regulation involves a high degree of sequence complementarity, and this was considered an indication that interaction of the Hfq protein would not be required [23, 45]. However, some researchers reported interference of Hfq in the *cis-encoded* ncRNA pairing [46-48]. In general, these ncRNAs act by complementing the mRNA ribosome-binding site, inhibiting, in turn, the translation [38, 45].

An example of cis-encoded typical ncRNA is the *5'ureB* of *Helicobacter pylori*, located 5'-antisense to the *ureB* gene that makes up the *ureAB* operon [49, 50]. This ncRNA contains 292 bp and negatively regulates ureAB operon expression by blocking translation in the 5 'portion of *ureB* (Figure 4). The *ureAB* genes of *H. pylori* are located in the cluster of two *ureAB-ureIEFGH* operons and encode the UreA and UreB subunits of apoenzyme urease. This enzyme is essential for the survival of H. pylori at low pH since its reaction produces NH3 and HCO3 into the environment, thus allowing homeostasis for bacterial growth [49]. The diversity of the cis-encoded ncRNA and their regulatory roles vary according to organisms as. For example, *Salmonella enteric serovar Typhimurium* possesses the cis-encoded ncRNA lesR-1 whose function is to control replication in eukaryotic cells [51] and *Salmonella serovar Typi* possesses the cis-encoded ncRNA AmgR involved in virulence in rats [52] and *AsdA* which regulates intracellular replication [53].

The same diversity observed with respect to regulatory strategies. In *Escherichia coli* the base pairing between the *cis-encoded* ncRNA GadY and its *gadXW* target mRNA causes the cleavage of the duplex between *gadX* and *gadW*, providing increased levels of the *gadX* transcript. The GadX product acts as a transcription factor for the *GadAGadB* operon during the synthesis of glutamate decarboxylase and this process consists of an acid stress deflating system in *E. coli* [6, 42].

The *SymR-SymE* system in *E. coli* consists of two genes, the cis-encoded ncRNA *symR* and the *symE* gene that encode a toxic protein [4, 6, 38]. The increase in the cellular concentration of the SymE protein decreases the synthetic activity of the ribosomes. The *cis-encoded* ncRNA symR negatively regulates the expression of the *symE* gene by the complementarity of ncRNA /mRNA bases, resulting in inhibition of mRNA*symE* translation and in the resumption of ribosomal synthetic activity [6, 38, 42, 50].

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Peng et al. (2000) [54] identified in the *Brucella abortus* 2308 the *cis-encoded* ncRNA BsrH that positively regulates the expression of the *hemH* gene, thus evidencing the importance of the regulatory expression that ncRNA BsrH exerts on its target mRNA.

The *cis-encoded* antisense ncRNAs are also common in the replication mechanism of plasmids. For example, in the replication control of the ColE1 plasmid, which uses ncRNA instead of proteins to initiate replication at the site of origin, two partially complementary RNAs are transcribed from opposite strands. The larger RNA, with 250-500 nucleotides (RNAII), is transcribed from the sense strand and forms a stable hybrid with template DNA. This hybrid is then processed by the RNAse H to generate a primer for the DNA polymerase. The smaller RNA, with 68-108 nucleotides (RNAI), is transcribed from the antisense strand and is complementary to the 5 'RNAII region. It functions as a negative regulator of primer formation by forming the duplex RNAI/RNAII that prevents the formation of hybrid RNAII/DNA template. The concentration of RNAI is proportional to the number of plasmids per cell, thus constituting a negative *feedback* loop that regulates plasmid replication in response to metabolic changes [37, 55].

2.2 *trans-encoded* ncRNAs

As previously mentioned, the mode of action of *trans-encoded* ncRNAs differs from cis-encoded ncRNAs by the limited sharing of base pairs with their target mRNAs [42, 50, 56]. This type of ncRNA is encoded in *trans* and may have target mRNAs at different locations in the genome. The transcribed ncRNA generally requires the chaperone Hfq protein to stabilize the target ncRNA-RNA interaction due to imperfect base pairing and thus avoids its eventual degradation by RNase [39, 42, 56, 57]. The most studied chaperone is the Hfq protein that in *E. coli* interacts with 40% of the ncRNAs [6]. Schoroeder *et al*. (2016) [58] report that almost 50% of all bacterial species possess *trans-encoded* ncRNAs that require the chaperone protein Hfq, one exception being *Listeria monocytogenes* in which most *trans-encoded* ncRNAs are independent of Hfq.

The interaction of Hfq with *trans-encoded* ncRNAs is involved in post-transcriptional regulation in several species of bacteria, and may exert a negative or positive effect on their mRNAs [59, 60]. The structure of Hfq, based on studies of crystallography, shows a hexameric protein, homologous to Sm proteins that have two motifs (Sm1 and Sm2) [39, 54]. In E. coli, Link et al. (2009) [61] characterized two RNA binding sites of the Hfq: proximal protein that

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binds to the ncRNA and target mRNA and the other distal one that binds to the poly U tail (Figure 5) [62].

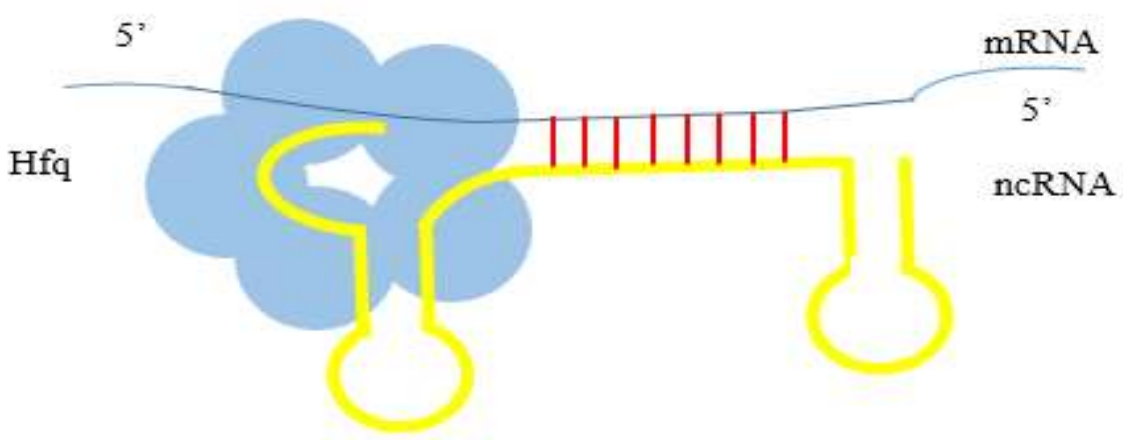

Fig. 5 - Probable model of interaction of Hfq protein with the ncRNA / mRNA pair.

Peng and collaborators (2015) [54] have reported, through fluorescence resonance energy transfer (FRET) studies, structural models in the Hfq interacts with PAPI, PNPase and RNaseE. Soper *et al*. (2010) [63] described three regulatory ncRNAs DsrA, RprA, and ArcZ in *E. coli* that regulate positively the translation of RpoS sigma factor when ncRNA and *rpoS* mRNA pairing occurs. They identified the formation of an inhibitory clamp in the 5'UTR region and demonstrated that binding to Hfq is important for the stability of the RNA: RNA complex. In the negative regulation, base pairing occurs between the ribosome binding sequence (RBS) and ncRNA, blocking the ribosome binding, or degradation by RNases.

In *E. coli*, regulation of OmpC protein expression involves the Micf ncRNA and a 5 'UTR regulation of 22 antisense nucleotides to *ompC* mRNA [64]. This interaction also involves Hfq and results in inhibition of translation. In *Salmonella typhimurium* is the Micc ncRNA, associated with the Hfq protein, which silences the *ompD* mRNA through the duplex of 12 base pairs RNA/RNA in the protein-coding region. MicC does not inhibit translation initiation in the downstream position, but it accelerates RNaseE-dependent activity [65, 66].

In *Salmonella enterica serovar Typhimurium* the *trans-encoded* ncRNA IsrM, controls the pathogenic factor SPI-1 [67]. In contrast, RybB-1 and RybB-2 are associated with regulation of oxidative stress response [68]. In *Salmonella enterica serovar Typi* the *trans-encoded* ncRNA RfrA and RfrB exert function in the regulation of iron homeostasis [69]. In Clamydia trachomatis the *trans-encoded* ncRNA IhtA exerts the function of inhibitor of histone Hc1

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protein, whereas in *Neisseria meningitidis* the *trans-encoded* ncRNA Nrrf has property of regulation of iron homeostasis [70-72].

Other examples of *trans-encoded* ncRNA: AbcR-1 and AbcR-2 in *Brucella abortus* are related to virulence in rats and are important for the survival of macrophages [73] and Mrc7 in the *Mycobacterium tuberculosis* species is involved in the regulation of the secretion system of TAT [74].

3. RIBOSWITCHES

Riboswitches are structured elements of non-coding RNA considered *cis-encoded* elements of RNA, located mostly in the 5'UTR region of a target mRNA and less frequent at the 3 'end UTR [7, 42, 50]. However, Loh *et al*. (2009) [75] described a case of riboswitch that controls *in trans* the expression of the virulence regulating protein PrfA in *Listeria monocytogenes*. They have the ability to control gene expression at the level of transcription and translation and to acquire different conformations in response to environmental signals, such as high temperatures and the binding of small molecules, such as metabolites or metal ions [9, 42, 76]. Recently, a wide variety of *riboswitches* have identified in prokaryotes. *Bacillus subtilis* has 2% of all its genes regulated by riboswitches that do not bind to intracellular metabolites, such as Flavin mononucleotide (FMN), thymine pyrophosphate, S-adenosyl-methionine (SAM), lysine and guanine (Figure 6) [43, 50, 77, 78].

The structure of the riboswitch is composed of two parts, the aptamer region that serves as the binding site for a ligand and the expression platform that assumes a suitable conformation for the transduction (Figure 7) [42, 79]. The binding of the binding molecule causes conformational changes in the native *riboswitch* structure, which can regulate transcription and translation processes [7]. The latter sequence of the expression platform forms a splicing with the aptamer domain, whether or not it is bound to the linker and may possibly signal a transcription term or control the helical structure at the ribosome-binding site [7, 80].

Thus, *riboswitches* used to regulate the termination of mRNA transcription (attenuation) and translation initiation [81]. In transcription when the ligand binds to the mRNA in the aptamer region, conformational changes occur, resulting in the formation of an alternative clamp (Figure 7a) [42]. This clamp acts as a transcription terminator that inhibits gene expression. Binding of the linker molecule to the alternative clamp leads to anti-termination (Figure 7b). Binding of a ligand that causes structural change and leads to sequestration of RBS

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prevents translation (Figure 7c). In contrast, binding of a linker may cause RBS exposure and promote translation (Figure 7d).

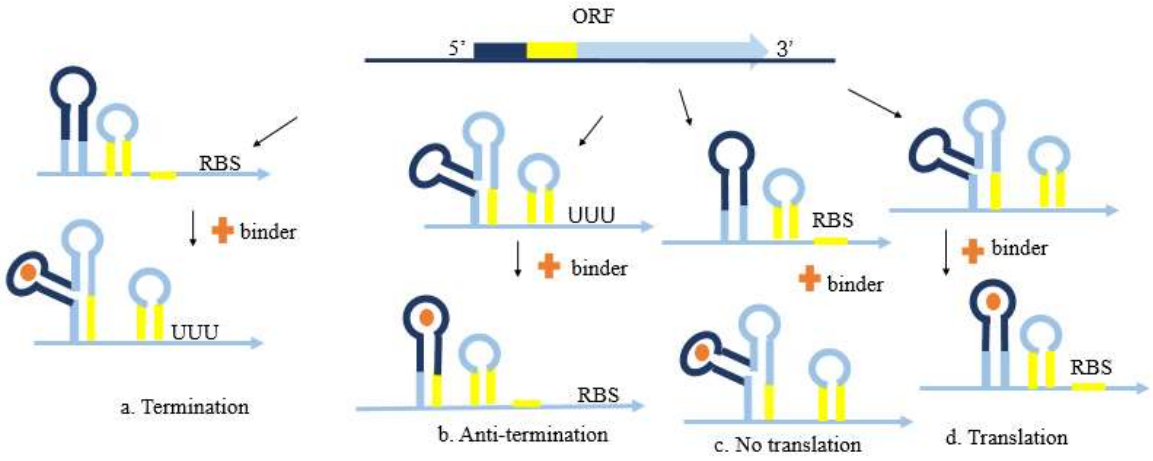

Fig. 6 – *Riboswitch* Structural Arrangement and Binding Regulatory Function. The aptamer region (pink) and an expression platform (yellow) on the 5' UTR of the respective mRNA (blue) form *Riboswitch*. Binding molecule has a regulatory function in the processes of transcription (a and b) and translation.

Riboswitches may play a role in biological cell systems because of the large number of gene families involved. Corbino et al. (2005) [82] found methA motifs in *Agrobacterium tumefaciens* similar to the S-adenosylmethionine decoding *riboswitch* (SAM). The SAM-II *riboswitch* class presents a great structural diversity, of low conservation, being able to alter the conformational structure of the mRNA [7, 83, 84, 85].

The structure of the *riboswitch* may contain several motifs that control gene expression by detecting specific metabolite concentrations, which makes these structures promising targets for antibiotics. Suresch *et al*. (2016) [86] have shown that S-adenosylmethionine riboswitch-III, present in anaerobic bacteria, is involved in the methionine and SAM biosynthetic pathway regulation process. In the presence or absence of the ligand, S-adenosylmethionine riboswitch-III exerts a double function, which facilitates the conformational change between the partially and fully folded state, forming a stable duplex structure, which strengthens the interactions between the Shine-Dalgarno nucleotides (SD) and anti-Shine-Dalgarno (aDS).

Perez *et al*. (2016) [87] validated, through an in vivo gene expression system, the cobalamin-*riboswitch* of the *cyneobacterium Synechococcus sp*. strain PCC 7002 and have

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proposed that methionine biosynthesis is probably the only means of using cobalamin in this strain of cyanobacteria.

4. The ncRNAs that Motivate Protein Activity

The first ncRNAs characterized in the *E. coli* enterobacterium include 4.5S, 6S, tmRNA (RNA transfer-messenger) and Spot42 [42, 88]. In *E. coli*, Spot42 RNA was discovered almost 40 years ago as a small and unstable RNA molecule encoded by the *spf* gene also present in *Vibrionaceae*, a class of γ-proteobacteria. *Salmonicidal Alivivium* encodes a Spot42 RNA ncRNA with 84% identity to that of *E. coli*. Deletion of the *spf* gene results in a 25% decrease in the action of RNA polymerase I (Pol I) [89]. This ncRNA optimizes carbon uptake and metabolism by binding to target mRNAs related to this metabolism, such as galactose operon *galK*. The ncRNA Spot42 is an example of ncRNA that acts traditionally by the ncRNA/mRNA interaction, but there are cases, such as the CsrB and the 6SRNA that regulate target protein activity and usually have specific recognition sequences [39].

The *E. coli* CsrB ncRNA has 22 GGA sequences considered binding sites for the CsrA protein [88]. By binding to the protein, it regulates mRNA stability and translation. The CsrA protein regulates carbon storage [90] through binding to mRNAs and is the essential component of the Csr regulatory system. [91] This system is responsible for the repression of a variety of stationary phase genes, negatively regulating gluconeogenesis, glycogen biosynthesis and catabolism, and biofilm formation. In addition, it activates glycolytic pathway, acetate metabolism and flagellum biosynthesis. CsrA acts post-transcriptionally repressing the gene expression of enzymes essential for the metabolism of carbohydrates such as ADP-glucose pyrophosphorylase (*glgC*), glycogen synthase (*glgA*), glycogen branching enzyme (*glgB*), glycogen phosphorylase (*glgP*). Thus, there is a decrease in the intracellular levels of biosynthetic glycogen enzymes and the intracellular decrease of glycogen synthesis [91]. CsrA destabilizes its target mRNAs by binding to the nucleotide-coding region of nucleotides -18 and +3, which includes (RBS), preventing translation of the mRNA and promoting its degradation by RNase. The csrB ncRNA competes directly with the target mRNA of CsrA and the dimeric protein CsrA recognizes the GGA motif of the structure of the nsRB ncRNA and the target mRNA. The CsrC and CsrB ncRNAs in *E. coli* modulate the activity of CsrA and the binding of the target mRNA to the protein [39, 42]. Thus, intracellular levels of CsrA also regulated by the CsrB and CsrC ncRNAs witch act as antagonists and capture CsrA. In *Erwinia spp* the RsmA protein, homologous to CsrA, regulates several genes involved in plant disease

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[92]. In *Pseudomonas aeruginosa* Csr (*Rms*) controls several systems like Rhl and several virulence factors [92].

Hindley identified 6S RNA in 1967 in *Escherichia coli* [25]. Since then, 6S RNA predicted through computational tools and experimentally analyzed in several bacterial species [93]. Wehner *et al*. (2014) [94] analyzed 1611 bacterial genomes and determined a set of 1,750 RNA 6S genes, 1,367 new. In the *Rfam* database, the 6S RNA described by two entries, the first RF00013 with 153 sequences, and the second RF01685 with 89 sequences.

It has been demonstrated in *E. coli* that 6S RNA (*ssrS* gene) can act in the transcription process by associating with sigma 70 factor dependent RNA polymerase (6S RNA:RNAP), modulating its function. This ncRNA sequestrates almost all of the RNAP holoenzyme in late stationary phase and thereby contributes to transcriptional adaptation of stationary phase growth [87, 93, 94].

Regarding the position of 6S RNA in the genome, a synthetic pattern observed for the *Bacteria* domain and this pattern is most widely observed in Gamma-Proteobacteria. Among the common synaptic genes are: *ygfA* (5-formyltetrahydrofolate cyclo ligase), *zapA* (ZapA protein), *AAA (+)* (ATPase superfamily) and peptidase_M24 (PF00557 family of metallopeptidases) [95, 96]. P. Gutiérrez, *et al*. (2005) [92] identified the ZapA protein in *Bacillus subtilis* together with the tubulin FtsZ protein.

5. CRISPR *(Clustered Regularly Interspaced Short Palindromic Repeats)*

In 2007, Barrangou [97] and colleagues demonstrated in bacterial cells the change in resistance to phage infection by removing or adding spacer sequences similar to the sequences of invading phages. This strategy defined an adaptive immune defense system that uses short RNA and called *Clustered Regularly Interspaced Short Palindromic Repeats* (CRISPR). In the genome, the CRISPR locus contains hundreds of spacer sequences (~ 26-72 nucleotides) flanked by repeats of 21 to 47 nucleotides. They are usually associated with proteins (*cas*), forming the CRISPR system, which confers RNA-mediated resistance against nucleic acids from, for example, bacteriophage, plasmids or mobile genetic elements [1, 43, 98, 99]. The CRISPR locus is present in ~ 40% of bacterial genomes and in 90% of archaeal genomes [42, 100, 101].

Most of the prokaryotes containing CRISPR have multiple groups 2 to 20 loci, each organized in tandem containing up to 100 identical repeats between 25 and 50 base pairs [10

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1]. Cas proteins provide the enzymatic machinery required to acquire new spacers and create recognition marks of invading elements [1029].

The arrangement and processing of CRISPR RNAs involves phases (Figure 7) [102]. Adhesion: invasive DNA is integrated into the CRISPR locus, resulting in new replicates in the matrix (Figure 7a); Expression: transcription of the transcript containing the phage RNA (new spacer) and its processing by the Cas proteins to form crRNA (matrix crRNA composed of single spacer sequences flanked by short repeats) and adjacent coded Cas proteins (Figure 7b); Interference: it is the formation of the complex CrRNAs Cas Cas proteins and later, the invasion happens to the target DNA and its respective degradation (Figure 7.c).

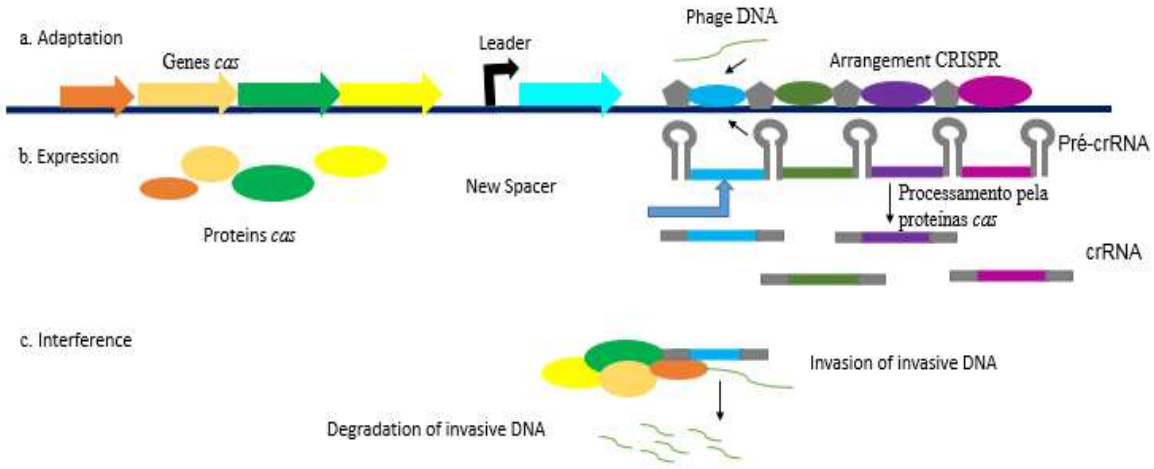

Fig. 7 - Array model of CRISPR system\Cas. The. Adaptation: DNA phage integrated into the CRISPR matrix and formation of the new spacer. B. Expression: Processing of cas proteins. W. Interference: Invasion to target DNA and its respctive degradation.

CRISPR may expressed in species such as *Legionella pneumophila*, where sRNA is the Cas2-dependent crRNA involved in the stress response [103-105]. In *Listeria monocytogenes* the RliB-CRISPR sRNA also makes use of the CRISPR\Cas system in antiviral resistance [106,107]. *Francisella novicida* possesses the ncRNAs, Cas9-dependent crRNA, tracrRNA and scaRNA, with CRISPR\Cas property in the regulation of viral endogenous factors [105]. According to Marraffini (2016) [108] the strain Streptococcus pyogenes has the CRISPR \ Cas9 system whose function is to intermediate the anti-phagocytic resistance. In *Campylobacter jejuni* PT14 the CRISPR\Cas14 system protects against invasion/presence of bacteriophage [109].

Amitai and Sorek (2016) [110] studied the mechanisms involved in the adaptation of CRISPR-Cas to the perception that the exogenous DNA once identified by the CRISPR/ Cas

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system is integrated into the genome of the host cell, thus generating immunological memory, a natural ability based on the information contained in the DNA. Such a system properly manipulated in the formation of new bases of information storage in living organisms, something that has investigated in recent years.

6. Prediction of ncRNAs and ats Targets

Bioinformatics is dedicated to the development of computer programs for the treatment of biological data and the identification of gene sequences, the prediction of three-dimensional protein configuration, the identification of enzyme inhibitors, the promotion of protein grouping, the establishment of phylogenetic trees and the analysis of expression experiments gene [111]. It provides tools for the development of Genomics, Transcriptomics, Proteomics and Metabolomics [112].

Regarding the prediction of ncRNAs, several computational approaches have developed for the identification of genes in intergenic regions of prokaryotic genomes [113, 114]. Many of these are based on the search for transcriptional signals, conserved promoter sequences, rho-independent terminator, transcription factor site, such as sRNAPredict [116], putative prediction algorithm analysis using TransTermHP database [116, 117] or TRANSFER [118]. sRNAPredict3 and SIPHT are recent computational versions of prediction of ncRNAs in bacteria) [16]. sRNAscanner and sRNAfinder [119] were developed to overcome the limitation of the prediction of available transcription signals in all genomic sequences and proved to be efficient.

The nocoRNAc (non-coding RNA characterization) is a computational tool developed to study the interactions between ncRNA-mRNA in conjunction with the prediction of ncRNAs in bacterial genomes. This program uses transcription terminator signals predicted through the TransTermHP tool, the promoters are identified by the *Stress Induced Duplex Destabilization* (SIDD) model and verifies possible regions to be destabilized [120, 121]. Thus, regions of the genome flanked by promoter sequence and Rho-independent terminator sequence, candidate to encode ncRNAs, identified. In a distinct approach, the *Cufflinks* tool locates regions of the genome with considerable levels of transcription and free ORFs [122].

Comparative genomic analyzes have been applied to predict new ncRNAs, and conserved sequences identified for the first time in the intergenic region (IGR), shared by clustering and comparison of multiple alignments are classified as ncRNAs. Programs such as QRNA [12], ERPIN [123]), ISI [13], INFERNAL [124], MSARI [125] and RNAz [126]) compared thermodynamic stability through prediction of conserved stable RNA structures, predicting

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ncRNAs in bacteria [14,127]. In the case of secondary structures, the RNAFold performs statistical statistical analysis of the RNA folding, through the perturbation of the thermodynamic parameters, for its prediction [119, 128, 129].

Regarding target prediction, the development of models is very important as it integrates bioinformatics for prediction and experimental validation for confirmation of mRNA targets. The classification of ncRNAs provides information on the complementarity of bases (perfect or imperfect) with their target mRNAs and eventual binding to proteins, altering their activity [130-134]. The targetRNA2 targeting mRNA prediction tool is one of the most widely used currently [135]. It utilizes various features including conserving ncRNA in other bacteria, the secondary structure of both the ncRNA and each target candidate, and the energy of hybridization between the interactions. It has the ability to integrate data from RNA-seq material when available. Another computational approach used to predict target mRNAs, the *Inta*RNA tool [136], is also considered quite efficient and rapid in predicting the interactions between ncRNA/ mRNA. It uses energy-free hybridization and has integration with the CopraRNA tool, which predicts ncRNAs by comparing the query-sequence with available sequences in the program [136, 137]. Other approaches include direct detection by means of *microarrays*, *northern blotting*, [3, 30, 111, 138, 139].

Pnek *et al*. (2008) [140] have identified predicted ncRNA in *Streptomyces* based on the study of sequence conservation in intergenic regions, localization of the transcription terminator factor, and genomic arrangement of syngenic genes to ncRNAs. They detected expression of 20 ncRNAs by *microarray* and RT-PCR, used a computational approach to determine secondary structure and identified 6S ncRNA. Voss *et al*. (2009) [141] used a cyanobacteria model to predict ncRNAs from transcriptome and proteome data and identified the Yfr2a-Yfr2c ncRNA, a conserved structure among cyanobacteria. To predict the existence of the 5'-operon-leader, the Rho-independent 3'-transcription terminator and riboswitches, these authors used the computational tools TransTermHP, ClustalW, RNAz, and RNAfold for validation using Northern Blot [142].

Modi *et al*. (2011) [137] performed the functional characterization of ncRNAs in *E. coli* through network inference, based on a compendium of gene expression profiles with functional prediction and on the regulatory interactions of ncRNAs. These authors validated experimentally the functions attributed to three ncRNAs, IsrA and GlmZ, involved in DNA damage response, and GcvB, involved in the regulation of amino acid availability. Khoo and collaborators (2012) [143] integrated several computational methods of prediction and analysis of ncRNAs and identified 29 in *Burkholderia pseudomallei* among which [8] were considered new. Ignatov and colleagues (2013) [144] used material and transcriptomic analysis to reveal that *Mycobacterium avium* and

M. tuberculosis contain different sets of ncRNAs in the intergenic regions and have suggested that this characteristic may be the basis of the observed physiological differences between the two species. Schroeder *et al*. (20) observed that in the genus *Rickettsia*, ncRNAs are the major post-transcriptional regulators involved in virulence, survival, plasmid expression, primary and secondary metabolism, and presumably encode trans-encoded ncRNAs involved in the pathogen interaction and host.

Conclusions and perspectives

Knowledge about the post-transcriptional regulation that ncRNAs exert on a given target mRNA related to its position and matched in ncRNA: mRNA, and which sRNA class being worked on. There are different classes of sRNAs and how these can regulate several mechanisms is forming regulatory network, which plays a fundamental role in the regulatory circuit of the genome under study. The advancement of computational technologies aiming at providing a knowledge about the role and functionality of non-coding RNA in the various domains of life. Which have developed research for prediction and validation that help the understanding of what considered genomic trash today it known of the great importance that ncRNAs exert despite size are excellent builders and foundations that make up the genomic machinery.

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