DOI: 10.4255/mcpharmacol.12.02

Small RNAs of Pathogenic Bacteria: Not Small Enough to be Overlooked for Therapeutics

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Abstract

The discovery of non-coding small RNA and miRNA has increased the understanding of gene regulation in all forms of life. Even though majority of research in the non-coding RNA field is devoted towards higher life forms, from prokaryotic point of view; bacteria also possess non-coding RNAs which are addressed as small RNA or sRNA. sRNA and miRNA may differ in biogenesis but the functional resemblance is unequivocal which well accords with the complexity of genome in which they reside. sRNAs have now been discovered in several prokaryotic genomes, many of which belong to pathogenic bacteria. Here we review the sRNAs found in pathogenic microorganisms with special attention to the sRNAs which play a role in pathogenicity and thus can be exploited for developing therapeutic approaches for controlling infection.

Keywords: Small RNAs; miRNAs; Multiple Drug Resistance; Antisense; Therapeutics; Pathogenicity

Introduction

The number of small RNAs (sRNAs) that have been discovered in the bacterial species has increased manifold in the past decade. Non coding small RNAs like micro RNAs have been implicated in mammals in several diseases and pathological conditions like cancer and diabetes (1). In contrast to the eukaryotic short interfering RNAs (siRNAs) and micro RNAs (miRNAs), which are antisense regulators sharing a common biogenesis and protein elements, the bacterial small RNA show high diversity in size and structures. Apart from sRNAs there are two more types of non-coding RNA; riboswitches and clustered regularly interspaced short palindromic repeats (CRISPR) RNA (2, 3). Riboswitches also modulate gene expression but are confined to untranslated regions of messenger RNA, whereas CRISPR RNA is involved in protection of bacteria and archea from foreign DNA and phages.

A class of non coding RNA is associated with Type I Toxin Antitoxin systems. Toxin antitoxin systems are genetic elements comprising of closely linked genes encoding for a toxin and a labile antitoxin which either renders toxin inactive or prevents its expression. RNA acts as antitoxin and regulates toxic peptide at post transcriptional level. Hok-Sok Type I TA systems were first discovered in R1 plasmids and were involved in post segregation killing (4). Chromosomal Type I Toxin Antitoxin systems such as TisB-IstR TA system localize at intergenic region (5), while TA systems like Ibs-Sib and Ldr-Rdl have been found amongst genomic repeats (6-8) and have been implicated in stress response (9).

The sRNAs are normally untranslated, vary in size between 50 to 250 nucleotides in length and are mostly involved in adaptive response to changes in the cellular milieu. The sRNA have been discovered in several pathogenic species of bacteria; *Mycobacterium* spp. (12), *Listeria* spp.(13), *Acinetobacter* spp. (14), *Salmonella* spp.(15, 16), *Vibrio* spp.(17,18), *Staphylococcus* spp. (19, 20) and *Pseudomonas* spp. (21-24). sRNA genes have been classically detected in the microbial genomes by a combination of bioinformatics tools followed by experimental confirmation using northern blot techniques. The sequencing of microbial genomes and their availability in public domains have aided the discovery of sRNA genetic loci tremendously. The bioinformatics search for sRNA genes begins by collating the sequences of conserved intergenic regions of the genome, followed by identification of orphan promoter and terminator sequences (2). Recently, the use of tiling arrays for whole genome expression profiling has obviated the use of computational approaches (25). Amongst bacteria,

Received 06/17/11; accepted 09/01/11
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80-100 sRNA species have been reported in *Escherichia coli* genome. Other species of bacteria seem to possess more number of sRNAs. Over all, it is estimated that bacterial species will have sRNA species of the order of hundreds per genome (26). Understanding the sRNA functions has been target of many research groups; taken together they have been found to regulate many important functions for the bacterial cell. sRNA have been found to be involved in regulating the growth, viability, heat shock, pH adaptation and virulence. Broadly, the sRNAs can be categorized into two groups – one which interacts with Sm-like molecular chaperone protein 'Hfq' and the other which does not. Hfq is an essential host factor for *E.coli* RNA bacteriophage Qβ replication. The hexameric form of the protein makes a ringed tertiary structure and stabilizes sRNAs by interacting with single stranded A/U-rich regions (10). In Gram negative bacteria, Hfq provides a podium for sRNA-mRNA interaction thus leading to regulation of the genes. Using the knockout version of bacterial strains, it has been indicated that the Hfq protein regulates the virulence of gram negative bacteria (11).

There are many number of sRNAs characterized in several bacteria, in this review we are describing sRNAs in bacteria (Table 1) with a major focus on sRNAs involved in virulence in some human pathogenic bacteria.

Small RNA of *Mycobacterium tuberculosis*: role in intracellular survival

Small RNAs in *Mycobacterium* spp. were identified using the cDNA libraries from low molecular weight *M. tuberculosis* RNA (12). Based on genomic location of clones with respect to ORFs and transcriptional orientation (antisense with respect to ORFs), nine sRNA B11, B55, C8, F6, G2, ASdes, ASpks, AS1726 and AS890 (Table 1) were found of which four (ASdes, ASpks AS1726 and AS890) were cis-encoded while the rest were trans-encoded. On Northern blot analysis, the sRNAs were found to be post transcriptionally processed and differentially expressed at exponential and stationary phase respectively. RLM-RACE (RNA ligation mediated RACE) and 5' RACE mapping revealed the presence of unique transcription start sites for four trans encoded sRNAs i.e. B11,B55,C8 and F6 while the rest of the sRNAs accommodate multiple 5'ends . Out of all sRNAs, only one trans acting sRNA B11 had its own transcription unit while the rest were co-transcribed from downstream or upstream genes. Of the nine sRNAs, one was found to be a mycobacterial homologue of the 4.5S RNA molecule which is involved in protein secretion by being part of signal recognition particle. Oxidative stress potently induced the expression of four sRNAs (B11, B55, F6, ASpks) which in fact, hint at the role of these small RNAs in pathogenesis as *Mycobacterium* spp. are exposed to reactive oxygen species in phagosomes of macrophages and neutrophils (12). On overexpression, two sRNAs B11 and G2 were found to be lethal (12). They were considered to functionally regulate the proteins involved in cell division since the cells exposed were elongated. The role played by the cis encoded sRNAs was deduced by the function of the cognate antisense ORFs. For two cis encoded sRNAs, ASdes and ASpks, the partners were fatty acid desaturase and polyketide synthase respectively. While the former is an essential gene for growth of *M. tuberculosis* the latter is implicated in bestowing antigenicity to the contagion. Both of the genes and their homologues are differentially expressed under pathogenesis and this can be attributed to the cis encoded sRNAs which are potent enough to act even at trans. Hence, the dynamism and complexity associated with *M .tuberculosis* infection could be accounted by yet to be discovered sRNAs encoded by its GC rich genome.

sRNAs of *Clostridium* spp.: role in toxin production and drug resistance

Clostridium genus represents organisms with potential pathological and biotechnological implications. Regulatory RNA in this genus control both virulence and metabolic pathways. A trans encoded sRNA VR-RNA (Table 1) in *Clostridium perfringens* mediates transcriptional regulation of two toxin genes *cola* and *plc* which codes for collagenase and alpha toxin respectively (27). Both of the toxins play a crucial role in the pathogenesis. VR-RNA up-regulates the expression of the toxin encoding genes *colA*, *plc*, *ptp* (protein tyrosine phosphatase), *cpd* (2'-3'-Cyclic nucleotide phosphodiesterase) and downregulates the *ycgJ*, *metB* (cystathionine gamma synthase), *cysK* (cysteine synthase) and *ygaG* genes (27). Furthermore, deletion studies indicated that the 3' region is important for VR-RNA regulatory activity. A recent study has indicated that VR-RNA up-regulates expression levels by bringing about stabilization of mRNA transcripts (Figure 1) (27, 28). The 3' regions of VR-RNA pairs with complementary sequence present in the leader sequence of *colA* mRNA and cleaves at position -79 and -78 from the

A of the initiation codon (ATG) (28). The processed transcripts were stable compared with longer intact transcripts and *colA* mRNA was found out to be labile in a VR-RNA-deficient strain and processed transcripts were undetectable. Complementation with 3' region of VR-RNA restored the expression of *colA* mRNA which further validates that up-regulation mechanism is brought about by the complementary base pairing in between the target mRNA and VR-RNA (28). Besides VR-RNA, RNomics has revealed a number of putative sRNAs encoding regions that are present in the sequenced genomes of the various *Clostridium* spp. Many of the predicted sRNAs were found to be a member of Rfam database and had either structural or functional role. In a recent study, a putative sRNA sCAC610 (Table 1) found in *Clostridium acetobutylicum* was able to up-regulate the expression of a putative ABC transporter CAC0528 which in turn can bring about clindamycin resistance (29). The expression profiling validated that both the sRNA sCAC610 and the Target CAC0528 expression were induced with increasing concentrations of clindamycin. The ABC transporter was conserved across all the clostridial genomes which highlighted its functional importance but the sRNA was only conserved in genomes of *C.botulism*, *C.acetobutylicum* and *C.beijerinckii* which relates well with the potency of clindamycin against genus *Clostridium*.

sRNA of *Salmonella* spp.: role in pathogenesis associated stress tolerance

Membrane homeostasis in *Salmonella typhimurium* is coordinated through joint efforts of sigma E and two sRNAs ; RybB and MicA (Table 1). Under envelope stress response (ESR), it was observed that after induction of *rpoE*, there was rapid reduction of the *omp* mRNAs (30). Since , there was no putative repressors which were activated by sigma E (30-32) and the fact that the *omp* mRNAs were unusually stable with a half life of seventeen minutes (33), rooted out any option hinting on transcriptional regulation. Furthermore, induction of ESR following mutations in a RNA chaperone protein Hfq (34, 35) highly endorsed the fact that the ESR is post transcriptional in nature. The post-transcriptional response through trans acting sRNA would also justify the rapid shutdown of outer membrane protein (OMP) synthesis upon envelope stress. Based on the above facts, sRNAs in *Salmonella* genome were screened for sigma E binding sites (36). The search yielded two sRNAs, RygB and MicA with perfect consensus sequences corresponding to Sigma E promoters. Further analysis, showed that these sRNAs were up-regulated along with *rpoE* and were not expressed in Δ*rpoE* strains. The sRNA, RygB regulated a wide range of OMPs which includes the most abundant OMPs of *Salmonella* i.e. OmpA, OmpC, OmpD and OmpF, with OmpC and OmpD showing maximum repression whereas MicA regulated only OmpA (36,37). In *rygB* deleted strains, membrane homeostasis was lost as demonstrated through elevated sigma E levels. Recent studies indicate that the conserved 5'UTR region in RybB, along with Hfq , transform into a multiple target binding domain which can bring about down-regulation of many mRNA through 7 base pair (bp) Watson-Crick pairing (38). Amazingly, only the first 16 nucleotides, termed R16, fused with random RNA are sufficient to bring about the down-regulation of Omp mRNAs (38). An R16 containing hybrid RNA was sufficient enough to respond to ECR and mediate the peculiar response. The RybB RNA when bound to the cognate target sequence in mRNA inhibited the formation of 30S-mRNA pre-initiation complex which in turn prevented translation initiation (Figure 2). The RybB target sequence position has been found to vary for different target mRNAs but generally it is more than 50bp upstream or downstream of initiation codon (38). For OmpN, OmpD, OmpS and OmpW the target sequence is just downstream of the initiation codon and any shifting of this target site with respect to initiation codon affects the regulation (39). For OmpA and FadL the target sequence was found to be present downstream of the fifth codon and this regulation required RNaseE (Figure 3). RNaseE, being an enzyme responsible for mRNA turnover, is involved in degradation of both sRNAs and sRNA-Target mRNA conjugates. Even though RNaseE mediated degradation of sRNA-mRNA conjugates can be assumed to be a part of RNA turnover process but recent co-immunoprecipitation experiments have revealed the presence of Hfq-RnaseE complexes (40). These experiments indicate the recruitment of RNaseE on Hfq-sRNA-mRNA conjugate or the dynamic association of Hfq and RNaseE for bringing about target mRNA degradation. Eventhough RNaseE could be dispensable in case of tranlationally inactive sRNA-mRNA conjugates (41), they are essential in cases where sRNA binds deep into coding sequence of the of the target mRNA (42).

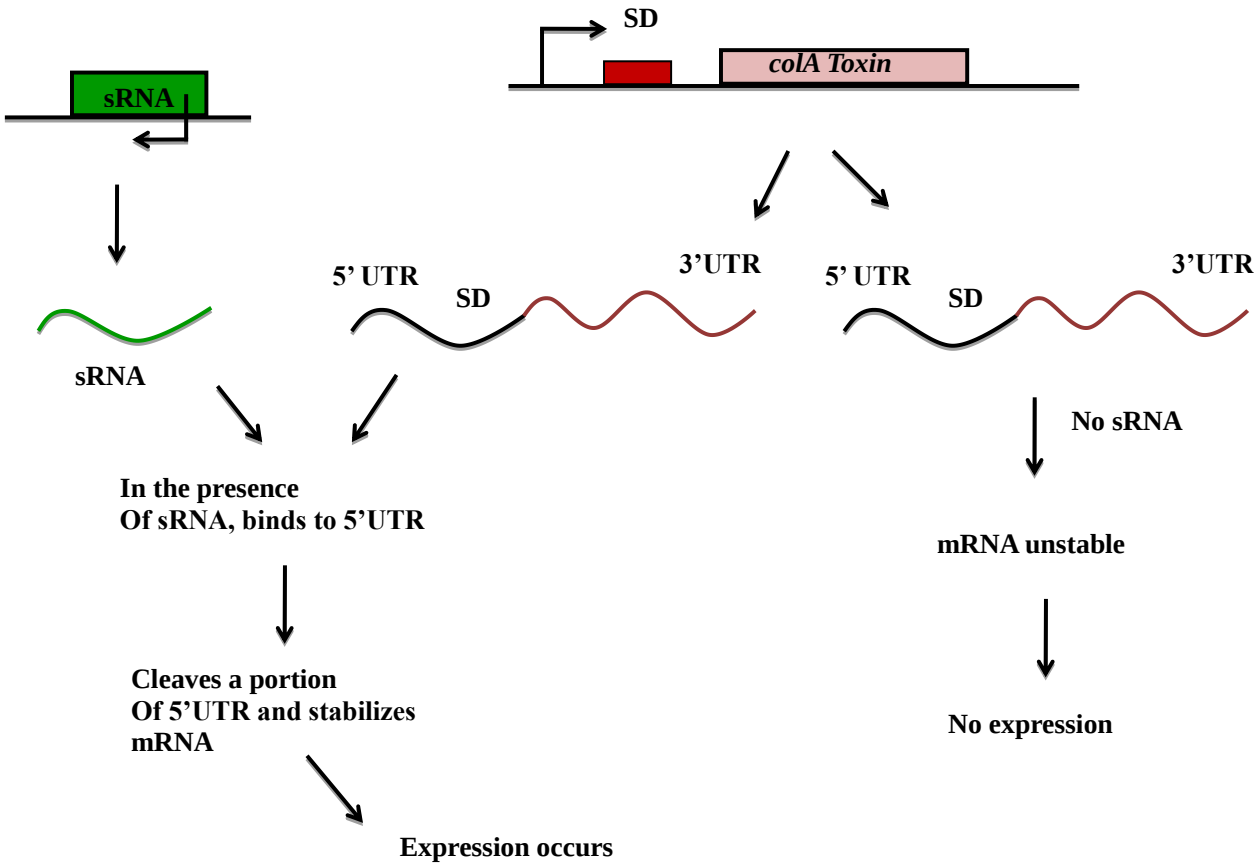

Figure 1. sRNA activating the expression of target genes by stabilizing the mRNA. The sRNA when bound to the 5'UTR of the target genes brings about the cleavage of some nucleotides on the 5'UTR which in turn stabilizes the mRNA and elevates the expression of the target genes.

Salmonella spp., as well as some other pathogens, owe their virulence to the pathogenicity islands found in their genome. The pathogenicity islands contain cluster of virulence genes and have also been found to encode sRNAs which play an important role in infection. RNomics and northern blot analysis revealed the presence of 19 sRNAs termed Island encoded small RNA (Isr) at pathogenicity islands (43). IsrC and IsrH were found to be up-regulated under most stress conditions including oxidative stress, osmotic shock, Iron depletion, pH stress, heat shock as well as in logarithmic and early stationary phase. IsrL and IsrB RNA were found to be up- regulated when the cells were grown in minimal media which indicates their role in nutritional stress. The sRNAs, IsrF and IsrI, were detected under both osmotic stress and stationary phase whereas IsrD & IsrN were detected at stationary phase only.Inside macrophages, along with OxyS sRNA, some other island encoded sRNAs were also upregulated. The OxyS sRNA along with Hfq RNA chaperone, down regulates the expression of the *rpoS* gene which encodes for sigma S subunit of RNA polymerase. The OxyS RNA mediates the repression of *rpoS* mRNA by base pairing at Shine-Dalgarno (SD) sequence and occludes translation initiation (44). The purpose of down regulating sigma S under stress conditions is to prevent redundant transcription of antioxidant genes by both sigma S and OxyR - a transcriptional factor generally induced by oxidative stress. Additionally, one of the sRNA, IsrJ was implicated in the invasion of the *Salmonella* spp. into nonphagocytic cells and it was found out that IsrJ plays a critical role in the injection of bacterial effector proteins into the cytosol of the host cells which paves way for *Salmonella* spp. invasion. Double mutants of *isrJ* and *hilA*, a central regulator of invasion, renders the bacterium incompetent of invasion (43). In conclusion; pathogenesis of *Salmonella tymphimurium* can be attributed to the dynamic regulation brought about by sRNAs.

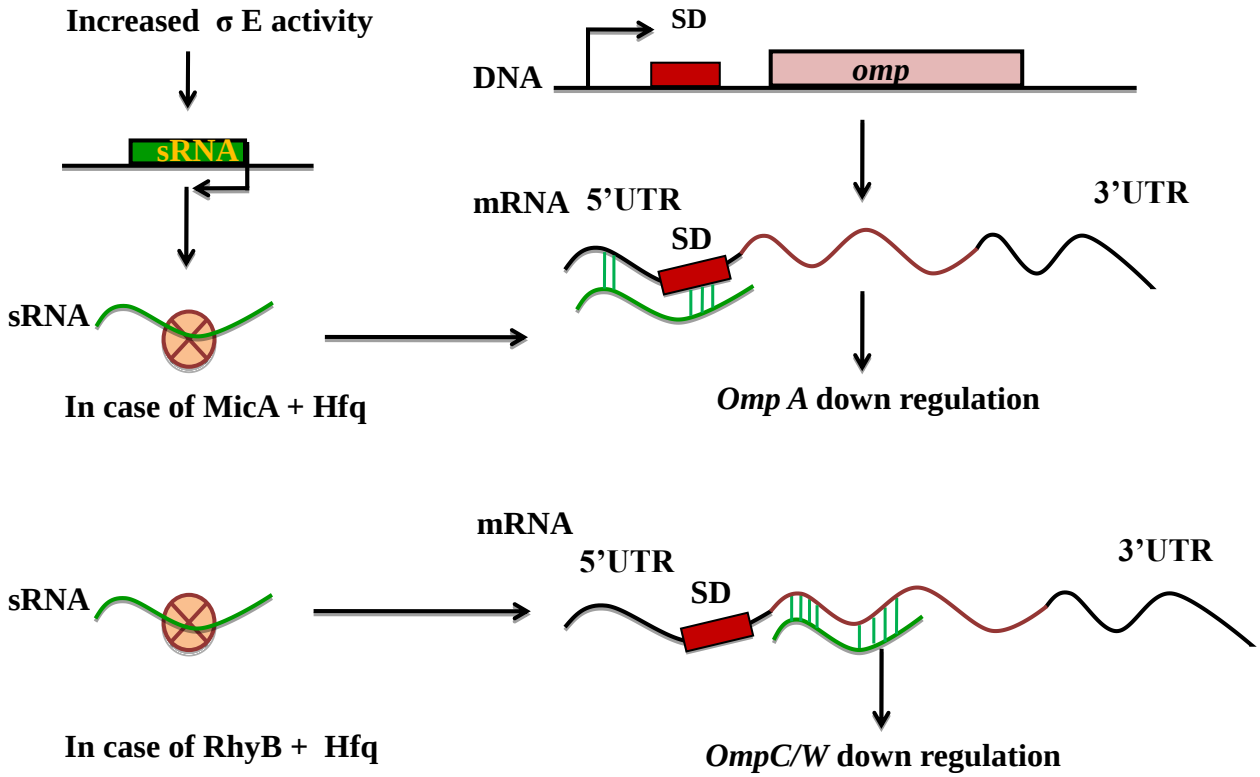

Figure 2. Regulation of OMPs by sRNA. Under stressed conditions σ E level increased inside the cell which ultimately increasesthe expression of two sRNAs, MicA and RhyB respectively. Hfq is responsible for the activity of MicA and RhyB small RNA. MicA with the help of Hfq binds to Shine Delgarno (SD) sequence of *omp A* mRNA and down regulates its expression. RhyB also acts in the same manner but it binds five codon downstream to the ATG and down regulates *omp N/W* mRNA expression. denotes Hfq.

sRNAs of *Listeria monocytogenes* : role in intracellular survival and neutralizing host defense

Listeria monocytogenes is facultative anaerobe, intracellular bacterium which is one of the most virulent food-borne pathogen. RNomics revealed presence of 150 sRNAs which were differentially expressed under extracellular and intracellular growth of *Listeria monocytogenes*. Out of 150 candidates, 88 belonged to trans acting putative sRNAs, 29 to antisense based sRNA and the rest to cis encoded sRNAs. The seven sRNA candidates Rli31, Rli33-1, Rli50, Rli78, Rli80, Rli105 and Rli112 (Table 1) were found to be differentially expressed during intracellular and extracellular growth. Under intracellular conditions, the expression of Rli3 and Rli33-1 was elevated whereas Rli112 and Rli78 expression levels were induced in extracellular conditions. Isogenic deletion mutants of the sRNA Rli31, Rli33-1 and Rli50 affected the pathogen's ability to propagate intracellularly as compared to the wild type strain (13).The expression levels of Rli50 were quite similar under extracellular and intracellular growth but the phenotype in Δ*rli50* strains was a contrast. This can be attributed to the simultaneous deletion of Rli112 during the deletion of Rli 50 since they share the same locus but are on opposite strands. LhrA, an Hfq dependant sRNA in *Listeria monocytogenes* is emerging to be a global regulator. It has been shown to alter the expression of approximately 300 genes. Recently, *chiA*, a chitinase encoding gene was found to be one of the targets directly regulated by LhrA. ChiA probably plays a role in pathogenicity since it mediates the degradation of glycoproteins on the host cells which might play a role in infection (13).

sRNAs in *Vibrio cholerae*: role in quorum sensing and virulence regulation.

Vibrio cholerae is a human pathogen and uses quorum sensing for pathogenicity and biofilm formation. In *Vibrio cholerae,* quorum sensing requires three signaling cascades and seven small regulatory RNA (three Csr sRNAs and four Qrr sRNAs) (Table 1). The VarS/VarA phosphorelay system has its role in *V. cholerae* pathogenesis, and

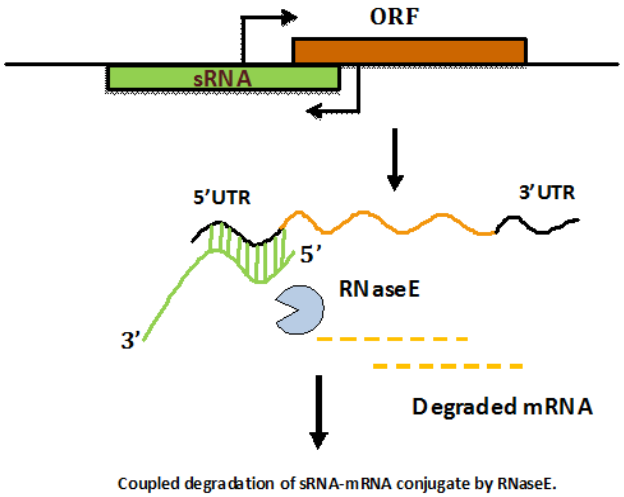

Figure 3. sRNA mitigating mRNA through RNaseE mediated degradation. Both cis and trans encoded sRNA, can downregulate the expression of target genes through enhanced degradation of corresponding mRNAs. These sRNAs, complementary to either 5' UTR, open reading frame or 3'UTR can bind cognate mRNA with or without the help of Hfq like RNA chaperone and initiate the degradation through RNase E. RNaseE being a part of general RNA turnover mechanism can initiate degradation of sRNA-mRNA conjugates irrespective of their translation status.

acts in parallel to the CAI-1-CqsS and AI-2-LuxPQ quorum-sensing systems (18).

In *Vibrio cholerae,hapR* mRNA encodes the HapR protein, the master regulator of quorum sensing cascade, had also been implicated in virulence. At low cell density VarS/VarA does not induce expression of CsrBCD small regulatory RNAs, CsrA (important post transcriptional regulator) protein remains active and promotes enhanced functioning of LuxO by an unknown mechanism. When LuxO gets activated, it activates the expression of four sRNAs Qrr1, Qrr2, Qrr3, Qrr4. These sRNAs with Hfq chaperone binds to Shine-Dalgarno sequence in the 5' untranslated region (UTR) of *hapR* mRNA and inhibits its translation. Absence of this transcription factor results in down-regulation of proteases such as HA/protease, up-regulation of virulence factors such as ToxT and formation of biofilms by enhancing expression of exopolysaccharide synthesis genes like *vpsk*, *vpsL* and *vpsQ* (45). At high cell density CsrB, CsrC, CsrD sRNAs get activated and sequester CsrA protein. This indirectly regulates the expression of Qrr sRNAs via LuxO. When the *qrr* genes are not transcribed, *hapR* mRNA is stabilized. HapR protein activates HapA which is a Zinc metalloprotease and is implicated in the entero-toxicity associated with cholera (46).

sRNAs in *Pseudomonas aeruginosa* : role in biofilm formation, virulence and quorum sensing

Pseudomonas aeruginosa is an opportunistic human pathogen. There are several sRNAs that have been identified from the genome of this bacterium (Table 1) and have been predicted to play a role in regulation of virulence by controlling quorum sensing and biofilm formation (21-24). Recently the regulatory mechanism of PhrS sRNA has been identified in *Pseudomonas aeruginosa*. PhrS regulates expression of many genes required for synthesis of virulence factor pyocyanin PYO (47,24). The genes for PYO (virulent factor) are under the regulation of a common transcriptional regulator PqsR. PqrR is synthesized by the induction of a co inducer 2-heptyl-3-hydroxy-4-quinolone (PQS) which is synthesized under environmental stress like increase in cell density or oxygen limitation. The expression of PhrS sRNA is under the regulation of an oxygen responsive regulator ANR (anaerobic regulation of arginine deiminase and nitrate reductase pathways) and the expression of PqrR is regulated by PhrS sRNA. An open reading frame (uof) of 40 amino acids is present in the 5' untranslated region of *pqsR* mRNA which is translationally coupled with *pqsR* gene(24). Under oxygen rich conditions, the translation of both *uof* and *pqsR* is abrogated by an inhibitory loop structure formed by *pqr* mRNA that sequesters the ribosomal binding site (RBS) for both the genes. When bacterial cells enter the stationary phase, they face oxygen limitation, during which ANR induces PhrS transcription. When PhrS is available in the cell it binds and open up the inhibitory structure at the *uof* RBS which in turn leads to the translation of *uof* along with *pqrR* (Figure 4). PqrR translation ultimately increases the translation of virulence factor PYO. Thus PhrS is the first small RNA which provides the link between oxygen availability and quorum sensing associated virulence (24).

sRNAs in *Staphylococcus* spp. : role in pathogenicity island gene regulation and toxin production.

Staphylococcus aureus have plethora of virulence factors encoded by its genome which is quite evident by the broad range of infections caused by it. But the shear presence of number of virulence factors also requires a dynamic genetic machinery to coordinate its expression. Indeed, *Staphylococcus aureus* had evolved the machinery comprising of global

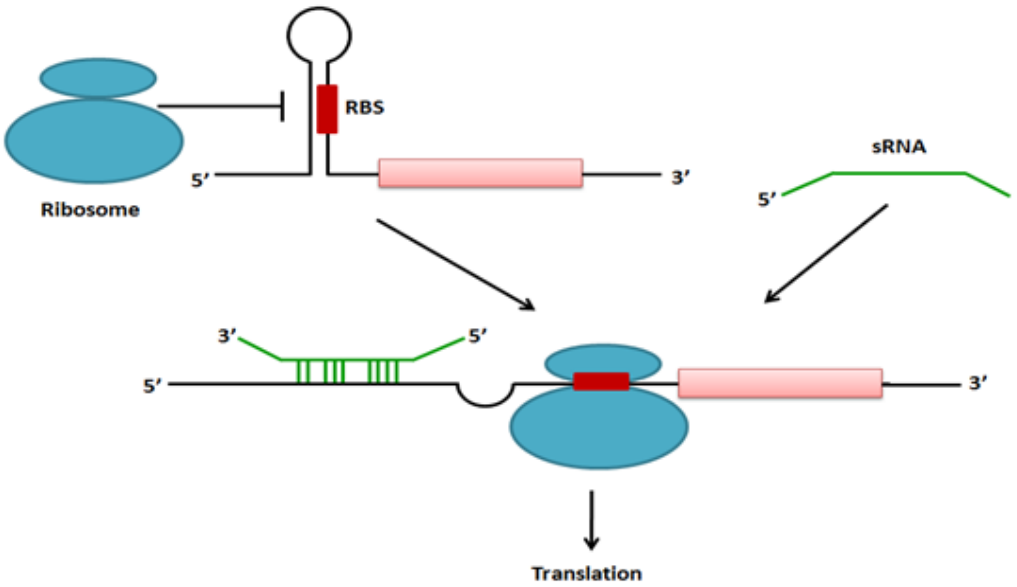

Figure 4. Activation of translation by sRNAs . Expression of some target genes is elevated through sRNA action. The 5'UTR of such genes constitutes a secondary structure which sequesters ribosome binding sites and abrogates translation. Upon binding of sRNA which is partially or fully complementary to 5'UTR sequence, the secondary structure is disturbed and the ribosome binding site (RBS) is exposed. Once RBS is exposed, 30S-mRNA initiation complex is formed and translation proceeds.

transcription regulators, quorum sensing components and sRNAs to coordinate virulence gene expression (48). RNAIII is a sRNA which had been implicated in regulation of virulence and multiple mRNA concerned with virulence. The RNAIII is a part of agr (accessory gene regulator) loci and it regulates the polycistronic mRNA RNAII coded from this loci. The RNAII encodes for the two component systemagrA, an intracellular effector molecule and agrC, agr signal responder. Alongside, RNA II also encodes for agrD and agrB. agrD encodes for a propeptide which is post translationally modified and processed and secreted by agrB , now termed as AIP (agr activating ligand is post translationally modified) which in turn activates the agrC (48-50).

Even though RNA III is cis encoded along with RNA II, they belong to a class of trans encoded sRNAs which regulates many mRNAs at post transcriptional level. Besides being a sRNA, it also acts as mRNA and codes for phenol soluble modulin peptides & Δ-haemolysin (50). RNAIII, as sRNA both down regulates as well up regulates expression of many genes. The 5' domain of the RNAIII interacts with the *hla* mRNA (encoding α-haemolysin) and activates its translation by preventing the formation of ribosome binding site sequestering intramolecular secondary structure (51). On the other hand, the 3'end and central domain of the RNA III which is quite conserved and repress the transcription of the early cell surface proteins, transcription regulators Rot which acts toxin repressor (52) and staphylocoagulase (53, 54). The repression is usually brought about by sequestering the ribosome binding site and preventing formation of ribosome initiation complex (54). RNAIII does not need any RNA chaperone for stabilizing its interaction which indicates that the tertiary structure is potent enough to establish stability while interactions.Besides, RNAIII, *Staphylococcus* spp. have copious amount of sRNAs which include cis-encoded sRNAs, pathogenicity island encoded sRNAs, cis encoded antisense RNAs and trans encoded sRNAs. The comprehensive details of these sRNAs have been reviewed recently (55).

sRNAs in *Acinetobacter* spp. : role in survival under nutrient stress

Acinetobacter genus includes highly MDR and pathogenic bacteria. Recently a sRNA, named Aar was identified in *Acinetobacter baylyi* by bioinformatics approach and was experimentally validated by northern blot analysis (14). This sRNA is unique in *Acinetobacter* spp. as it shows 98% sequence similarity with different strains of *Acinetobacter baumannii* (SDF, AB3070294, AB0057, ACICU, AYE and ATCC 17978). This sRNA is flanked by *trpS* encoding tryptophanyl-tRNA-synthetase II and *sucD* encoding succinyl-coA-synthetase subunit α genes. Aar sRNA up-regulates genes involved in amino acid metabolism and early expression of Aar sRNA was observed under excess of sodium chloride and during iron depletion

Table 1. Small RNAs involved in Pathogenicity and Virulence

S No.	Small RNA	Organism name	Function (target genes)	References
		sRNAs in Gram Negative genera		
sRNAs in *E coli*				
1.	MicF	*E coli*	Repression of porin synthesis(*ompF*)	61,62
2.	MicC	*E coli*	Repression of porin synthesis (*ompC, ompD*)	63
3.	MicA	*E coli*	Repression of porin synthesis (*ompA, ompW*)	64-66
4.	RybB	*E coli*	Repression of porin synthesis (*ompC, ompW*)	67,68
5.	RseX	*E coli*	Repression of porin synthesis (*ompC, ompA*)	69
7.	CyaR	*E coli*	Repression of porin synthesis and group behavior (*ompX, luxS, nadE*)	70-72
8.	OmrA	*E coli*	Repression of outer membrane protein synthesis (*cirA, fecA, fepA, ompT, gntP ,ompR*)	73.74
9.	OmrB	*E coli*	Repression of outer membrane protein synthesis (*cirA, fecA, fepA, ompT, gntP , ompR*)	73.74
10.	GcvB	*E coli*	Repression of peptide transport (*oppA, dppA,cycA, sstT*)	75-77
11.	RydC	*E coli*	Repression of putative ABC transporter (*yejABEF*)	78
12.	MgrR	*E coli*	Repression of LPS modification gene (*eptB, ygdQ*)	79
13.	SgrS	*E coli*	Protection against glucose-phosphate stress (*ptsG*)	80,81
14.	GlmZ	*E coli*	Induction of GlcN-6-P synthase (discoordinate regulation of *glmUS* operon)	82-84
15.	Spot42	*E coli*	Repression of galactokinase blocks degradation (discoordinate regulation of *galETKM* operon)	85
16.	GadY	*E coli*	Activation of acid response (*gadX*)	86,87
17.	RyhB	*E coli*	Iron-sparing(*sodB, sdhC, frdA*, activates *shiA*)	88-90
18.	FnrS	*E coli*	Repression of unneeded enzymes (*sodB, maeA, gpmA, folE, folX*)	91,92
19.	OxyS	*E coli*	Repression of unneeded activities (*fhlA,yobF-cspC, ybaY wrbA,rpoS*)	93,94
20.	ArcZ	*E coli*	Activation of σS; repression of alternative activities (*rpoS, sdaCB, tpx*)	95,96
21.	DsrA	*E coli*	Activation of σS; repression of hns (*rpoS,hns*)	97-100
22.	RprA	*E coli*	Activation of σS(*rpoS*)	101,102
23.	DicF	*E coli*	Inhibition of cell division (*ftsZ*)	103-105
sRNAs in *Salmonella typhimurium*				
24.	RybB	*Salmonellatyphimurium*	Repression of porin synthesis (*ompC, ompW*)	36
25.	ChiX	*Salmonella typhimurium*	Repression of chitoporin synthesis (chiP) (also *dpiBA*)	15
26.	CsrB	*Salmonella Typhimurium*	Protein sequestration (CsrA protein), virulence	16
27.	GcvB	*Salmonella typhimurium*	Repression of peptide transport (*oppA, dppA, gltI, livK, livJ, argT*)	106
sRNAsin *Bordetella pertussis*				
28	BprJ	*Bordetella pertussis*	Role in virulence (BvgA/BvgS system)	108
sRNAsin *Pseudomonas*spp.				
29	PrrF1 or PrrF2	*Pseudomonas*	Iron metabolism	21

		aeruginosa	(*sodB, sdhD, bfr*)	
30	RsmZ/RsmB	*Pseudomonas aeruginosa*	Protein sequestration (RsmA protein)	22,23
31	Phrs	*Pseudomonas aeruginosa*	Regulation of transcription factor (PqsR)	24
31	RsmZ/RsmY/ RsmX	*Pseudomonas fluorescens*	Protein sequestration (RsmA protein)	109,110
sRNAsin *Vibrio cholerae*				
32	Qrr1–Qrr4 sRNA	*Vibrio cholerae*	Inhibition of translation and mRNA degradation (*hapR*)	17
33	CsrB/CsrC/ CsrD	*Vibrio cholerae*	Protein sequestration (CsrA protein),virulence	18
sRNAsin *Acinetobacter baylyi*				
34	Aar	*Acinetobacter baylyi*	Up regulation of amino acid metabolism genes (*fadA, ilvI,ppC, glnA, serC, leuC*, and *gcvH* mRNAs)	14
sRNAs of Gram positive genera				
sRNAsin *Listeria monocytogenes*				
35.	RliD	*Listeria monocytogenes*	Repression of translation of PNPase	107
36.	RliE	*Listeria monocytogenes*	Repression of translation of *comC*, *comEA, comFA* and *lmo*0945	107
37.	RliH	*Listeria monocytogenes*	Repression of translation of *lmo1050* gene	107
38.	RliI	*Listeria monocytogenes*	Repression of *lmo1035* and *lmo 10136* gene	107
39.	LhrA	*Listeria monocytogenes*	Role in pathogenesis by targeting *chiA*gene	13
40.	Rli33 &Rli33-1	*Listeria monocytogenes*	Intracellular invasion	13
sRNAsin *Mycobacterium tuberculosis*				
41.	ASdes	*Mycobacterium tuberculosis*	Regulate fatty acid desaturase genes *desA1* and *desA2*	12
42.	ASpks	*Mycobacterium tuberculosis*	Regulate polyketide synthase gene *pks12*	12
43.	B11	*Mycobacterium tuberculosis*	Prevent growth	12
44.	G2	*Mycobacterium tuberculosis*	Prevent growth	12
45.	F6	*Mycobacterium tuberculosis*	Prevent growth	12
sRNAsin*Clostridium* spp.				
46.	sCAC610	*Clostridium acetobutylicum*	Plays role in drug resistance	29
47.	VR RNA	*Clostridium Perfringens*	Virulence (secreted toxins)	27
48.	VirX	*Clostridium Perfringens*	Virulence (secreted toxins)	111
sRNAsin*Staphylococcus aureus*				
49.	RNAIII	*Staphylococcus aureus*	Activation of translation (Hemolysin)	19,20
sRNAsin *Bacillus subtilis*				
50.	RatA	*Bacillus subtilis*	mRNA degradation (*txpA*)	112

conditions (14). The expression of sRNAs under harsh conditions indicates that it might have a role in survival and virulence of *Acinetobacter* spp. under these conditions.

miRNA & prokaryotic sRNA : a comparative outlook

Among the many functional sRNAs in eukaryotes, microRNAs and siRNAs are indeed the major players and anelaborate genetic machinery has evolved for their biogenesis as well as for facilitating their action. Genes encoding miRNAs are present in the coding as well as non-coding genes and they account for around 1% of the genes found in higher forms of life and in turn regulate 20%-30% of the genes (56). Biogenesis of the miRNA (57) occurs in the form of hair pin loop, termed as pri-miRNA, which is then trimmed by nuclear located dsRNA specific endonuclease Drosha enzyme to generate a 70bp imperfect stem-loop structure named pre-miRNA with an overhang of 2 nucleotides at 3'end. Through exportin-5, the pre-miRNA complex is transported to cytoplasm where it is recognized by the dsRNA specific endonuclease dicer through its PAZ (Piwi/Argonaute/Zwille) domain. Dicer mediates the cleavage of pre-miRNA into short imperfect dsRNA duplex, which is further recognized by RNA induced silencing complex. Based on the stability of the 5' end of the miRNA, functional miRNA strand is selected which implicates in the sequence specific targeting of the cognate mRNAs. Majority of the miRNA targets 3'UTR which is in contrast to sRNA which rather prefer to target 5'UTR and translation initiation region (57). Analogous to some sRNAs, miRNAs can target multiple mRNAs and can also bring about positive gene regulation. Transcription of miRNA has been coupled to external & internal environment which is sensed through suitable signal transduction systems (58) and in certain cases, the miRNA genes were transcribed in polycistronic fashion (59). As the sRNAs found in pathogenicity islands are transcribed as a cluster and have related functions, the same can be explained for miRNA gene clusters also. In short, despite of well understood difference in the biogenesis, biochemical machinery and mode of action between prokaryotic sRNAs and eukaryotic miRNAs is similar as both of them bind to the ends of the cognate target mRNAs (to 3' end for miRNAs and to either 3' or 5' for sRNAs). Also, broadly speaking sRNA and miRNA carry out functions in somewhat related manner at least at post-transcriptional regulation level and the differences they have, is just a reflection of the prokaryotic and eukaryotic genomic as well as biochemical complexity.

Conclusions

Since sRNAs have been implicated in pathogenesis of various microorganisms, it is tempting to think that modulation of their action can be explored for drug discovery and development. Discovering the chemical matter which can specifically enter into microbes and target specific small RNA can be a difficult task. On the other hand the target sRNA action can also be regulated by developing suitable drug delivery systems which are specific for an infection so that the sequence specific antagonists like peptide nucleic acids (PNA), morpholino oligomers or antisense RNA molecules could be delivered. With the threat of multiple drug resistant bacterial strains becoming eminent, we need immediate measures to counteract them. Even though the field of drug discovery has revolutionized with the advent of combinatorial chemistry, computer aided drug designing and high throughput screening; the discovery of new drugs is still limited by the reduced availability of new molecular targets. The popular antibiotics in use or in the pipe line for near future mostly target cell wall, cell membranes, DNA replication, transcription or translation. With sustained use of these drugs, there are chances that bacteria might be able to evade them as they have done with previous antibiotics of same classes. sRNAs regulate several proteins implicated in bacterial pathogenesis. Understandably, these proteins can be exploited as new targets for drug discovery. A case in example can be taken of the porins. Porins have been found to be regulated by sRNAs in some pathogens; one can start a reverse chemical genetics approach to find appropriate inhibitors to attenuate these proteins. Similarly, one can target the sRNAs and proteins involved in the secretion systems of the pathogens which can abrogate toxin production.

Gram-negative bacterial pathogens are getting noticed for development of multiple antibiotic resistance. There is not a single late stage drug in offering which specifically targets this category.Around 60% of the bacterial infections are due to Gram-negative bacteria (60). The Hfq chaperon protein is exclusive to Gram-negative bacteria and interacts with as much as 25% of the total sRNAs of a the bacterium. Knock out studies of Hfq have shown the important role this protein and

sRNAs play for the infection. Debilitating the Hfq and Hfq like RNA chaperones can yield broad spectrum antibiotics. While, targeting proteins like Hfq, one should keep in mind that the mammalian homologue of the protein (Sm family proteins) does not get affected by this approach.

Even though, one can hypothesize that sRNAs are likely to play a major role in development of multiple drug resistance, only a couple of small RNA had been correlated with development of drug resistance phenotype experimentally. Yet the above observations do assert on the need of future research on role of sRNAs in Multiple Drug Resistance (MDR) development. Another direction of interest can be to study the modulation of antibiotic action in MDR strains by attenuation of sRNAs responsible for cell envelope protein regulation. It will be interesting to see whether the sRNA over-expressed or debilitated strains of MDR bacteria can be better targets for antibiotics?

Acknowledgements

V. Rajesh Iyer and Rajnikant Sharma are supported by CSIR and MHRD fellowships of Government of India respectively. Naveen K. Navani and Ranjana Pathania are supported by grants from the National Agricultural Innovation Project (NAIP) of Indian Council of Agricultural Research (Grant number C4-30032).

Conflicts of Interest

No potential conflicts of interest to disclose.

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