Non-coding RNA Research 4 (2019) 86–95

Contents lists available at ScienceDirect

Non-coding RNA Research

journal homepage: http://www.keaipublishing.com/ncrna

On a stake-out: *Mycobacterial* small RNA identification and regulation

Sagarika Taneja, Tanmay Dutta*

RNA Biology Laboratory, Department of Chemistry, Indian Institute of Technology Delhi, Hauz Khas, New Delhi, 110016, India

ARTICLE INFO

Keywords:
Small RNAs
Base pairing
Antisense
Anti-antisense
Gene regulation by sRNA

ABSTRACT

Persistence of mycobacteria in the hostile environment of human macrophage is pivotal for its successful pathogenesis. Rapid adaptation to diverse stresses is the key aspect for their survival in the host cells. A range of heterogeneous mechanisms operate in bacteria to retaliate stress conditions. Small RNAs (sRNA) have been implicated in many of those mechanisms in either a single or multiple regulatory networks to post-transcriptionally modulate bacterial gene expression. Although small RNA profiling in mycobacteria by advanced technologies like deep sequencing, tilling microarray etc. have identified hundreds of sRNA, however, a handful of those small RNAs have been unearthed with precise regulatory mechanism. Extensive investigations on sRNA-mediated gene regulations in eubacteria like *Escherichia coli* revealed the existence of a plethora of distinctive sRNA mechanisms e.g. base pairing, protein sequestration, RNA decoy etc. Increasing studies on mycobacterial sRNA also discovered several eccentric mechanisms where sRNAs act at the posttranscriptional stage to either activate or repress target gene expression that lead to promote mycobacterial survival in stresses. Several intrinsic features like high GC content, absence of any homologue of abundant RNA chaperones, Hfq and ProQ, isolate sRNA mechanisms of mycobacteria from that of other bacteria. An insightful approach has been taken in this review to describe sRNA identification and its regulations in mycobacterial species especially in *Mycobacterium tuberculosis*.

1. Introduction

The genus *Mycobacterium* generally consists of nonpathogenic environmental bacteria, which are closely related to the soil bacteria *Streptomyces* and *Actinomyces* [1]. However, multiple clinically relevant highly successful pathogens like *Mycobacterium tuberculosis, Mycobacterium leprae*, and *Mycobacterium ulcerans*, the causative agents of tuberculosis, leprosy, and Buruli ulcers, also constitute the *mycobacterium* genus. Genus *Mycobacteria* are classified into three classes: *M. tuberculosis* complex, *M. leprae*, and non-tuberculosis mycobacteria [2]. Profound insight into this genus can reveal plethora of information about various kind of organisms, and can offer the connecting links between them. More than 140 species are known in the *mycobacteria* genus [3]. Infectious species like *M. tuberculosis*, *M. leprae*, *M. bovis BCG* etc. in this genus have attracted enormous interest of the scientific communities and the healthcare sectors.Successful infection of these microorganisms partially lies in their ability to escape the macrophage engulfment and successful proliferation inside macrophages [4,5].Identification of non-tuberculosis mycobacteria is intricate since they are diverse in growth temperatures, growth rates, and drug susceptibility, as well as in clinical relevance [1].

M. tuberculosis alone is contemplated as one of the most successful pathogens for causing tuberculosis in human and animals. It is the predominant causative agent for death than any other microorganism, and furthermore, > 30% of the current population of the world is believed to carry this bacterium in its non-proliferative state [6]. Interestingly, the development of effective drugs against this pathogen have always been a challenge because of its distinctive life cycle.Between two of its metabolic states, one is the latent persistent and other being the active replicative state [7]. For the development of drugs against *M. tuberculosis*, it is crucial to apprehend how does it switch between its two states, and on what factor does this transition depends? Initiation of the infection happens through an asymptomatic latent form, and a small number of individuals develop progressive lung disease. Occurrence of tuberculosis depends on the capability of pathogens to balance the altered environments within the host, and under proper circumstances, the transmission of signals that subvert the immune response in order to cause localized immunopathology [8,9]. Continuous macrophage activation is necessary to preclude reactivation of the infection [10,11]. *M. bovis* and five other mycobacterial species are also

Abbreviations: RBS, Ribosome binding site; TIR, translation initiation region; SD, Shine Dalgarno sequence; TF, transcription factor; UTR, untranslated region; RNAP, RNA polymerase; sRNA, small RNA; nt, nucleotide; ORF, open reading frame; CDS, coding sequence; IGR, intergenic region

* Corresponding author. RNA Biology Laboratory, MS 731, Department of Chemistry, Indian Institute of Technology Delhi, Hauz Khas, New Delhi, 110016, India.
E-mail address: dtanmay@chemistry.iitd.ac.in (T. Dutta).

https://doi.org/10.1016/j.ncrna.2019.05.001
Received 31 December 2018; Received in revised form 30 April 2019; Accepted 12 May 2019
Available online 16 May 2019

categorized in *M. tuberculosis* complex based on their high genome sequence similarities. Although these species have high resemblance in their virulence properties, but the host and phenotypic expression differ across species [12,13].*M. leprae* infection on the contrary develops leprosy, a disease with high inconsistency in clinical symptoms. Tuberculoid type leprosy is associated with granuloma formation and severe nerve damage as a consequence of killing of Schwann cells. In contrast, leprosy in multibacillary condition is recognized with heavily infected and inflamed perineurium [14].A fair extent of effort has been given to develop effective drugs against these microorganisms, however, little attention have been paid to understand how different regulators fine tune various physiological processes of these species right after the initiation of transcription. Small RNA (sRNA) regulators frequently modulate numerous posttranscriptional processes to alter gene expressions and cellular phenotype [15] and thus, the understanding the role and mechanism of these regulators will help manipulate mycobacterium virulence.

Aside from sRNAs, other classes of regulatory RNAs like CRISPR (clustered regularly interspaced short palindromic repeats) RNAs [16,17] and riboswitches [18,19] are also found in mycobacterial species. However, the functions of riboswitches or CRISPR will not be discussed in this review. Primary focus of this review is to comprehensively present an insightful analysis of how different strategies were taken to identify mycobacterial sRNAs and how these sRNAs act *in vivo* to interact with their targets and subsequently, modulate downstream gene expression.

2. Small RNAs and Hfq in pathogenesis

Numerous physiological circuits in bacteria are regulated by sRNA to remodel their gene expression in response to changing environmental conditions [20–27]. These sRNAs are usually 50–400 nt long and act as a linchpin for the regulation of gene expression under virulence, stress, quorum sensing etc. [23,27–29]. They are either expressed from the cognate genes or generated from the nucleolytic processing of larger transcripts [20].Enormous studies on the identification of sRNAs and their functional role in the cell have largely been investigated in *Escherichia coli* and *Salmonella sp*. Majority of the sRNAs in these species have been recognized with specific functions. Based on the analyses, small RNAs have been found to be synthesized either as *cis* or *trans* relative to their target mRNAs and generally regulate their expression by base pairing mechanisms resulting in altered mRNA translation and stability [30,31].*Cis*-encoded sRNA genes lie in the DNA strand, complementary to one from which target mRNA is transcribed, and hence, a broad region of complementarity is present between them. In contrast, *trans*-encoded sRNAs are transcribed from cognate genes, located far away from their target genes, and thus, share limited complementarity with their targets.

In majority of the instances, *trans*-encoded sRNAs require the assistance of a chaperone like Hfq or ProQ for a stable sRNA:mRNA base pairing [21,27]. RNA chaperone proteins also play a necessary role of protecting sRNA from cellular degradosome. Interestingly, mycobacterium species have been reported to be devoid of Hfq and ProQ-encoding genes in their chromosome [32], which warns to investigate how sRNAs in mycobacterium species acquire stability inside the cells. Although a wide array of sRNA regulators modulate their target gene expression by base pairing, a small number of sRNAs directly bind and regulate their function. These kind of sRNAs often mimic the structure of the proteins' cognate targets and sequester such RNA binding proteins. The protein once sequestered, it is no longer available to exhibit its specialized function on their targets [33,34].

Discoveries of increasing number of sRNA-mediated regulation in bacteria have intensified the interest to detect the potential link between the regulatory sRNAs and bacterial pathogenesis. Furthermore, RNA binding proteins like Hfq [32], CsrA [35] etc. have been implicated in impaired bacterial virulence [36]. RNA chaperone Hfq in gram negative bacteria including pathogenic species is essential to promote interaction between trans-encoded sRNAs with their targets. In those cases, pathogenic strains lose their virulence character upon deletion of *hfq* gene, presumably due to loss of sRNA function [32]. However, Hfq function in gram positive bacteria is ambiguous, as some of the pathogenic bacteria lack *hfq* gene in their chromosome [37,38]. This apprises the question of how is base pairing of *trans*-acting sRNAs in mycobacteria with their targets achieved and what would be the subsequent fate of sRNA-mRNA duplex. Probable alternatives are either mycobacterial sRNAs can independently interact with their cognate mRNAs without the assistance of Hfq or they possess different RNA chaperone, which has not been discovered yet. Recently reported additional RNA chaperones like ProQ in *Salmonella typhimurium* and Rv2367, a homologue of *Sinorhizobium meliloti* YbeY protein etc. [39,40] are also absent in mycobacteria, thus, it is not unlikely that mycobacterium will be evolved with a novel RNA chaperone.Nearly 70% of mycobacterial genome comprises of GC sequence, and hence, it is conceivable that the secondary structures of mycobacterials RNAs are highly stable owing to their higher degree of intramolecular stability due to high GC content. Moreover, the presence of intrinsic terminator and high GC content restrict the prevalence of AU-rich stretch, which are typical site for Hfq binding [41].

Rapid regulatory circuits particularly regulatory RNA networks in pathogenic bacteria are requisite in the changing environmental conditions for their adaptation, which eventually control their virulence. Small RNAs associated with regulatory proteins and two-component systems integrate environmental signals into essential outcome indispensable for bacterial pathogenesis [42]. Small RNAs have several advantages over protein transcription factors (TFs) in a regulatory circuit of a cell. Lower energy cost of sRNA synthesis, as they are much smaller than mRNA and do not generally require to be translated(27, 31), rapid binding to their targets, and faster clearance while they are no longer needed, have established sRNAs as better contender for controlling bacterial gene regulation.

Direct involvement of sRNAs in regulating bacterial pathogenesis stems from the fact that the deletion of sRNA genes resulted in the phenotype with impaired virulence [43,44]. A reasonable number of sRNAs have been implicated in bacterial virulence and it is growing with new discoveries. Functionally redundant quorum regulatory sRNAs, Qrr1-4, in *Vibrio harveyi* use distinct regulatory mechanisms to control four different target mRNAs; luxM, luxO, luxR and aphA. All of these sRNAs are the members of the quorum-sensing regulatory circuit [45,46] and thus, directly regulate the virulence character of *Vibrio sp*. One of the small pathogenicity island RNAs SprD in *Staphylococcus aureus* down regulates the expression of Sbi immune-evasion molecule, impairing both the adaptive and innate host immune responses [47]. Tn-seq transposon screening and targeted genetic approaches demonstrated that majority of the validated 56 sRNAs in *Sreptococcus pneumonia* have been implicated in important global and niche-specific roles in virulence [48]. Regulatory sRNAs, RivX and FasX,in *Sreptococcus pyogenes*have been demonstrated in virulence gene regulation and interactions with host cells, respectively [49].

A novel*cis*-encoded antisense small RNA 5'-UreB-sRNA in *Helicobacter pylori*, a human stomach colonizing pathogen, down regulates the expression of gastric acid acclimatization operon *ureAB*by truncation at neutral pH, but in acidic environments it releases its control to promote survival [9,50]. Small RNAs like RprA, DsrA, ArcZ, in *E.coli* and *Salmonella sp*. act as secondary regulator of bacterial pathogenesis by modulating the expression of stationary phage specific σ^S-factor RpoS [51,52], which transcribes many of sRNAs related to bacterial virulence. RNA thermosensor in *Listeria monocytogenes*, a bacteria with low GC content, has been widely established as one of the key regulators of bacterial virulence [53]. Recent reviews [48,53–56] on sRNAs will enumerate different types and mechanisms of sRNA to regulate bacterial virulence. Discoveries of new sRNAs in different pathogenic microorganisms are extending the list of sRNAs implicated in

pathogenesis.

3. Identification of sRNAs

Gene-encoded sRNA was first identified in 1984 through the discovery of a 174 nt long MicF RNA in *E. coli* [57]. However, first dedicated attempt for the identification of sRNA in bacteria through genome-wide analysis using systematic computational approach was carried out by different groups in 2001 to hunt for RNA secondary structures, orphan promoter and terminator sequence in the intergenic region in *E. coli* [58–61]. Small RNA list is extending with advanced technologies like deep sequencing, high density tilling microarray etc. [62,63]. In recent years, sRNA identification in large scale has been accomplished by a method, RNA-interaction by ligation and sequencing (RIL-seq), which discovered an extensive network of RNA-RNA interaction [64]. A single bacterium can possess hundreds of sRNA upon encountering to external cues. A handful of sRNAs in different microorganisms, largely from enterobacteria *E. coli* and *Salmonella*, have been characterized. Hence, the necessity to perceive sRNA function is heightened in bacteria, especially in pathogenic bacteria.

Arnvig et al. first identified a set of nine putative sRNAs in *M. tuberculosis* apart from their house keeping sRNAs like M1 RNA, tmRNA, 4.5S RNA etc. by screening cDNA libraries prepared from low molecular weight RNA fraction. Clones of sRNAs were validated by northern blotting and were further mapped for their mature 5' and 3' end [65]. In addition to the sRNAs transcripts from IGR (*trans*-encoded), ORF was also found to be the precursors sRNA transcripts (*cis*-encoded).All of the identified sRNAs in *M. tuberculosis* are predicted to be folded in a stable structure with a C:G ratio > 1. *M. tuberculosis* sRNAs possess C-rich stretches [66] in their structures, which presumably have enormous significance for interacting with the target RNAs in absence of Hfq [67,68]. A combined computational and experimental approach was utilized to identify sRNAs from *M. tuberculosis* and/or *M. smegmatis* and revealed that the expression of many sRNAs are conserved across the mycobacterial species [69]. A study on the total transcriptome of *M. tuberculosis* uncovered an abundance of noncoding RNAs including antisense transcripts, intergenic sRNAs and *cis*-regulatory elements [70]. Another automated approach using RNA-seq technology had been followed to globally identify the sRNA-encoding genes in *M. tuberculosis* [70]. Computational predictions from the sRNA identification protocol using the algorithm of high-throughput technologies (SIPHT) identified 144 sRNAs from *M. bovis* BCG and 34 of them validated by northern blotting. A combination of SIPHT and large scale northern blot validation additional 17 sRNAs from *M. bovis* BCG and 23 sRNAs from *M. smegmatis* were identified [71]. In the similar direction, transcriptome generated from an exponential phase culture was utilized for identification of 192 novel candidate sRNA-encoding regions in IGR. Additional 664 RNA transcripts, which are synthesized from the region complementary to the ORF by transcription, were also recognized and 28 sRNAs among them were validated by northern blotting [72]. *M. leprae* genome was also investigated for sRNA expression by whole genome tilling microarray [73].

A novel strategy was implemented by Li et al. [74] for the identification of novel sRNAs by expressing a heterologous RNA chaperone Hfq from *E. coli* in *M. smegmatis* with the aim that sRNAs especially *trans*-acting sRNAs in *M. smegmatis* will be enriched by Hfq interaction. Immunoprecipitation of Hfq divulged the presence of 12 *trans*-encoded and 12 *cis*-encoded sRNAs in the immune-precipitated fraction and many of those sRNAs were found to be differentially expressed at exponential compared to stationary phase, which indicates the involvement of sRNAs in the regulation of mycobacterial growth. Intriguingly, phylogenetic conservation analysis showed that five among these *cis*-encoded sRNAs are related to mycobacterial virulence. Although this approach explored the advantage of intrinsic sRNA-binding capability of Hfq, however, nonexistence of Hfq or any of its homologue in mycobacterium species limits the practicability of this approach [74].

4. Mode of regulation by sRNAs

Although a huge number of sRNAs have been identified and validated in mycobacteria, only a handful of them have been designated with their *in vivo* function [75]. Small RNA-mediated regulation of gene expression is largely achieved through base pairing mechanisms. Structural features of these sRNAs comprise of at least a single-stranded stretch with certain degree of complementarity with their target mRNAs for effective base pairing and a Rho-independent terminator, possessing a stable stem-loop with a poly(U) rich extension [76]. In a number of examples, base pairing of sRNA with its target mRNA is initiated by a fast high affinity contacts through a few exposed nucleotides in the seed region of the single stranded stretch of the stem-loop structure. Subsequent base pairing is assisted by initial “kissing” interaction through the rearrangement of sRNA secondary structures [28]. This type of structure-driven base pairing mechanisms are widespread among *cis*-encoded sRNAs. In contrast, interaction between *trans*-encoded sRNAs with their target RNAs in bacteria is primarily promoted by RNA chaperone [77,78]. However, mycobacteria lacks any homologue of Hfq or ProQ, and thus, how *trans*-acting sRNAs in mycobacteria interact with their cognate targets is still elusive and an intriguing area of active research.

4.1. Base pairing mechanism

The outcome of sRNA interaction with their targets is either the repression or the activation of target gene expression. Any sRNA pairing at the ribosome binding site (RBS) on mRNA will interfere 30S ribosome loading at the translation initiation region (TIR) on mRNA, which will ultimately inhibit the initiation of translation, the rate-limiting step in bacterial protein synthesis mechanism [79]. Majority of the sRNAs in bacteria have been reported to be induced in altered environmental conditions and occludes TIR. Intercession of ribosome binding mediated through stress induced sRNAs also delineated to promote Rho-dependent transcription termination [80]. sRNA:mRNA pairing surrounding 5'UTR or RBS or downstream coding sequence (CDS) recruits ribonucleases for rapid turnover of both target and sRNA [81] (Fig. 1A). Hfq furthermore protects sRNA, especially *trans*-acting sRNAs, from ribonuclease-mediated decay [82,83]. Contrarily, a class of sRNAs can anneal to the complementary sequence stretch of RBS or 5'UTR, which under normal condition is sequestered in a secondary structure, and liberate RBS for active translation by ribosome. Unfolding of this intrinsic inhibitory structure on mRNA extricate SD region for 30S ribosome binding for protein synthesis [84,85] (Fig. 1B). A number of sRNA base pairing to either 5'UTR or CDS protects and stabilizes mRNA from ribonucleolytic degradation, thus promoting active translation [86,87]. A set of sRNAs follow a general mechanism of the sequestration of RNA binding proteins’ which culminates in the elimination of their regulatory functions [88–90] (Fig. 1C).

Adaptation of microorganisms to altered environmental conditions is an inherent feature and plays a decisive role in pathogenesis. *M. tuberculosis* encounters a series of unfavorable conditions like abrupt change in temperature and pH during transmission between the host and external environment, exposure to reactive oxygen and nitrogen species as an outcome of the oxidative burst after being taken up by mammalian phagocytic cells, necessary nutrient and cofactor deprivation, iron homeostasis etc. Adaptation of *M. tuberculosis* to aforementioned stress conditions implicates the integration of regulation of protein transcription factors with RNA based regulatory network. Initial investigations recognized an analogous set of sRNAs in the transcriptome of *M. tuberculosis*, which indicates the potential role of these sRNAs in mycobacterial pathogenesis [91,92].

Conservation of sRNAs in distantly related species is rare. However, several ubiquitous sRNA regulators like 6S RNA, DsrA, RyhB etc., participate in analogous regulatory network under similar environmental cues [44]. Studies on *M. tuberculosis* sRNAs confirmed the existence of

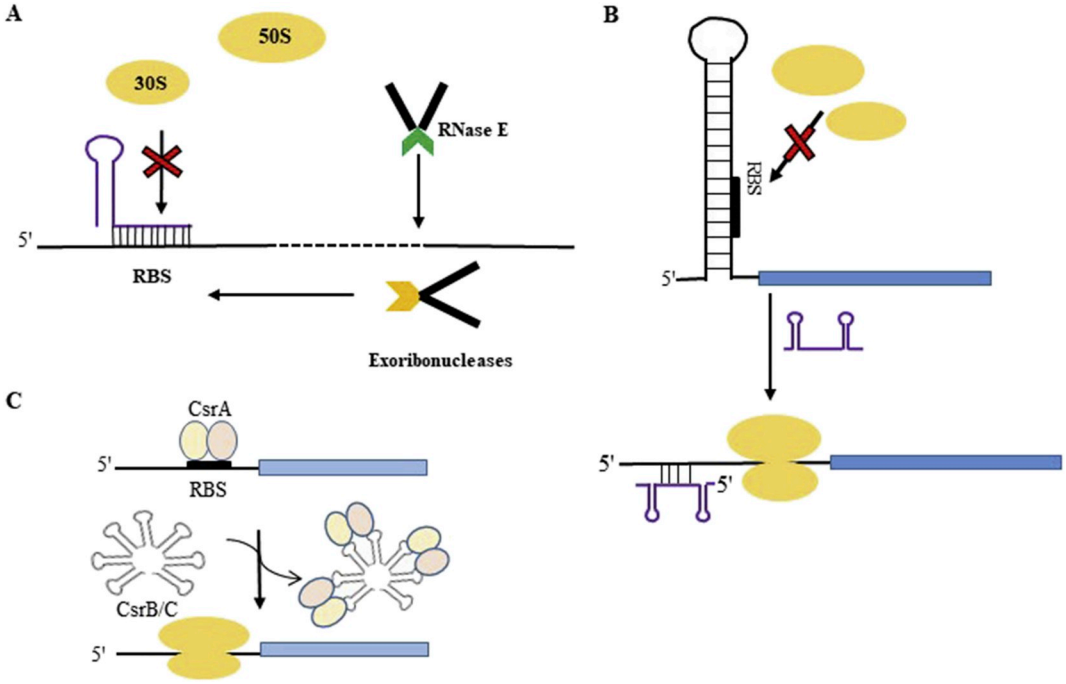

Fig. 1. Modes of sRNA action. General mechanism of repression by antisense pairing at RBS (A), active translation by anti-antisense pairing (B) on target mRNA. (C) Protein sequestration to regulate mRNA translation.

major housekeeping sRNAs. For instance, C8 RNA in *M. tuberculosis* is the essential housekeeping 4.5S RNA, a component of signal recognition particle [93]. Arnvig et al. first discovered the existence of sRNAs in *M. tuberculosis* [65]. ASdes and ASpks have been identified as sRNAs, which are *cis*-encoded to their target genes, *desA1* and *pks*, both of which have been described to regulate the lipid metabolism. The gene of DesA1, a fatty acid desaturase enzyme, is essential for *M. tuberculosis* growth [94] and is highly upregulated after being taken up by host macrophages during infection [95]. ASdes also exhibits substantial complementarity to another desaturase (DesA2), and thus, has enormous potential to behave as *cis*-acting regulator to *desA1* and *trans*-acting to *desA2*mRNAs [65]. Another *cis*-encoded sRNA ASpks is transcribed as an antisense RNA within the *pks12* gene encoding polyketaid synthase (Pks) 12. Pks is involved in the synthesis of mannosyl-β-1-phosphomycoketide, an immunogenically potent phospholipid component on mycobacterial cell wall, which is recognized as antigen by mammalian CD1 restricted T cell [96]. ASpks also shares significant complementarities to the mRNAs of other ketosynthase domains, and thus, possesses the ability to act as dual function sRNA [65].

A starvation induced sRNA, ncRv12659, in *M. tuberculosis* has been reported [97] to be synthesized from the complementary strand of Rv2660c mRNA. Transcription of ncRv12659 sRNA starts within region of PhiRv2 prophage and interestingly, is abandoned only in the strains that have retained PhiRv2. Accumulation of ncRv12659 culminates during *M. tuberculosis* infection. Hence, higher expression of this sRNA bestows a potential biomarker for tuberculosis and also for identification of the cells undergoing nutrient and oxygen starvation. Overexpression of ncRv12659 develops impaired cell growth as well as perturbation in the transcription profile of *M. tuberculosis* H37Rv. Additionally, the expression of more than fifty genes were affected and majority of them were upregulated. It has also been found that 5'-end of ncRv12659 act as a useful marker for phenotypic analysis of *M. tuberculosis* during infection with PhiRv2 positive strain [97]. A different sRNA Mcr11 in both *M. tuberculosis* and *M. bovis* with the transcription origin between *Rv1264* and *Rv1265* genes exhibited differential expression under conditions associated with the status of host macrophage and granulomas during infection [69,98]. *Rv1265* is a cyclic AMP induced gene and is upregulated both in *M. tuberculosis* and *M. bovis* during macrophage infection [99,100]. Hence, Mcr11 has got particular attention due to its link with cAMP metabolism [101]. The correlation between the growth dependent expressions of Mcr11 and upregulation during host macrophage infection clearly suggest the involvement of this sRNA in the progression of *M. tuberculosis* infection. However, the mechanism how Mcr11 operates in the cell is yet to be investigated.

Lack of any report regarding the presence any RNA chaperone in mycobacterium species has made the context very difficult to design a strategy to identify potential targets of *trans*-encoded sRNAs and to speculate their regulatory mechanisms. Phenotypic change as a consequence of the deletion of sRNA gene may reveal an alternate root for target identification. Overexpression of three trans-encoded sRNAs, F6, B11 and G2, showed intense effect on mycobacterial growth. Expression of both B11 and G2 under strong *rrnB* promoter [102] turned out to be detrimental for *M. tuberculosis* cell growth, while the outcome of F6 expression was exceptional slow growth of the cell. Strikingly, *M. smegmatis* upon transformation with B11-harbouring plasmid was grimly affected in their cell division and morphology [65]. The mechanisms of how these *trans*-acting sRNAs functions to regulate physiological process are yet to be investigated. Expression of another *trans*-acting sRNA, MTS2823, although abundant in all growth phases of *M. tuberculosis* cell, is markedly elevated either at the stationary phage of the cells or during infection condition [70]. Higher than the normal expression of this sRNA in the exponential phase caused downregulation of global expression of the genes related to exponential growth. Among the extraordinarily affected genes, methyl citrate network specific genes like *prpC* in particular and *prpD* are remarkably downregulated, which suggests the preferential targeting to minimize either the utilization of propionyl-CoA and/or oxaloacetate or the build-up of toxic intermediate like methyl citrate [70]. MTS2823 is not a bona-fide 6S RNA homologue but it exhibits functional similarities to 6S RNA. It accumulates up to 16% of the 16S rRNA level in lungs of mice during acute tuberculosis infection. MTS1338, also a member in *trans*-encoded sRNA list of mycobacterium, is highly elevated in the stationary phase of cell growth under the control of hypoxia responsive DosRS two component transcriptional regulator.

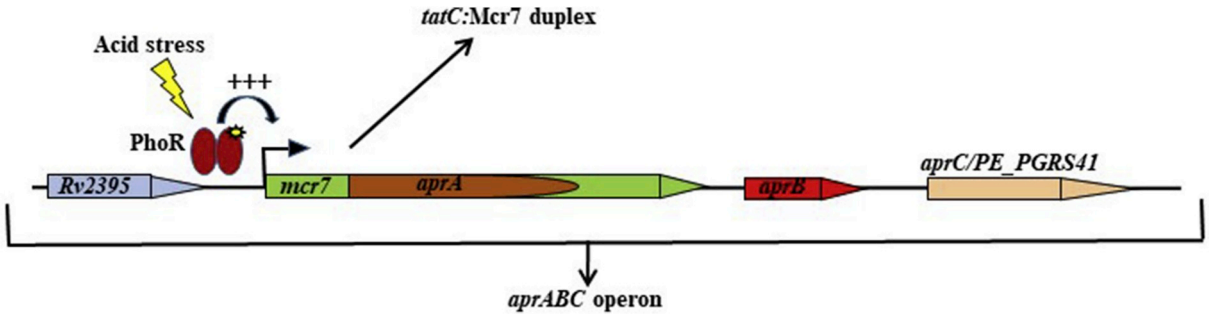

Fig. 2. *M. tuberculosis mrc7* and *aprABC* operon locus. The figure represents associated components of PhoPR-regulated operon and Mcr7. Acid-stress induction of AprA happens through a suggested mechanism of *tatC* and Mcr7 interaction.

Complete understanding of the regulatory function of a sRNA in mycobacterium was first accomplished by Solans et al. [103]. A PhoP regulated expression of a novel 350 nt long sRNA Mcr7 [69] in *M. tuberculosis* was observed from the IGR between *rv2395* and *PE_PGRS41*. PhoP is one of the components of PhoPR regulatory system [104], which is an essential factor for *M. tuberculosis* virulence where it regulates expression of nearly 2% of the genes including the genes involved in ESX-1 secretion apparatus, a crucial determinant for virulence [105]. PhoP protein, a part of PhoB/OmpR subfamily, was investigated by chromatin immunoprecipitation followed by high throughput sequencing for the identification of potential genes, which are regulated by this protein. PhoP was found to be capable of binding as much as 35 different loci on the *M. tuberculosis* genome. Mutation of *phoP* gene resulted in impaired production of pathogen specific cell wall components and slow growth [106]. *PE_PGRS41* gene, which is associated with the IGR carrying *mcr7* gene (Fig. 2), was found to be highly deregulated in *phoP* knock out strain. Among mycobacterial species the expression of *mcr7* was found to be prominent particularly in *M. tuberculosis* and its expression profile coincides with the proposed evolutionary pathway of the tubercle bacilli [107]. A highly structured fold with a 33 nt predicted free loop of Mcr7 was reported to interact with the 5'-end of *tatC* mRNA, encoded protein of which is a member of Twin Arginine Translocation (Tat) protein secretion apparatus [106]. This posttranscriptional interaction covers the region of putative RBS and first six codons of *tatC* mRNA. Thus, an antisense mechanism is followed by Mcr7 RNA to occlude RBS and subsequently to downregulate of *tatC* gene expression (Fig. 3). A transmembrane protein, encoded by essential *tatC* gene in *M. tuberculosis* [108], is a component of TatABC general secretary apparatus, which is indispensable for twin arginine motif containing protein export in their signal peptide [109]. Deletion of *phoP gene*, which lacks *mcr7* expression, resulted in the enrichment of the Tat-dependent substrates in the secretome. Reestablishment of plasmid vector-dependent *mcr7* expression in *phoP* mutant restores the secretion of Tat-dependent proteins, which emulate the wild type expression level. Complementation of *mrc7* alone in *phoP*-deleted strain did not reconstitute the wildtype virulence [106].

A novel DosR regulated 109-nt long sRNA DrrS in *M. tuberculosis* has been reported to be unusually stable with a half-life of several hours [110] due to its stable stem loop structure at its 5'-end. However, the addition of two or more unpaired nucleotides at the 5′end of that loop drastically decreases its stability (Fig. 4A). Accumulation of this sRNA is slow but strong from the onset of the stationary phase of growth. DrrS expression in the exponential phase of cell growth is low (< 1 copy per 10 cells) [6,70]. Extraordinary stability of DrrS play prime role in its accumulation in the stationary phase. Nitric oxide stress, a known condition for DosR induction, was found to upregulate *drrS* expression in a dose-dependent manner. DosR, which is induced by infection-associated stresses, is a response regulator of the two-component DosRS system [111]. Other stress conditions like oxidative stress, DNA damage, alteration in pH did not cause any change in the expression of *drrS*. However, DrrS appeared to be expressed at elevated level during chronic mouse infection [65]. Transcription of DrrS happens from two start sites: a weaker one at T1960601 and a stronger at A1960667. Transcriptional analysis reveals that DrrS transcription primarily happens from strong promoter site. Predicted structure of DrrS contains a short five base pair GC rich stem loop at 5′-end and a long imperfect stem with a short CU or CUC tail at its 3′-end. DrrS is transcribed as a precursor (> 300 nt) molecule (DrrS+) with extra residues at the 3′-end. Maturation of DrrS involves a rapid 3′ processing of a longer transcript, which lacks a canonical intrinsic terminator structure (Fig. 4B). DrrS + climaxes at early stationary phase and $DrrS_{108}$ accumulates continuously for three weeks into stationary phase [110]. Differential expression and considerable size differences of DrrS+ and mature DrrS signify that they might have divergent role at different phases of growth.

An in-depth characterization of mycobacterial regulatory sRNA in iron (MrsI) reveals that MrsI or ncRv11846, which is induced under multiple stress conditions like iron starvation, oxidative stress and membrane stress, interacts directly with its target mRNAs encoding nonessential iron-containing proteins to repress their expression [112]. MrsI is ~100 nt long highly structured sRNA with a predicted rho-independent terminator at the 3'-end and it is highly conserved across the Mycobacteriaceae and Nocardiaceae. It has been experimentally validated that MrsI directly base pairs through a 6-nt seed region in its 5'-end with the 5'-UTR of one of its target mRNA *bfrA* and upregulates its expression during iron starvation. Knockdown of *msrI* gene [113] in *M. tuberculosis* genome culminates in enhanced expression of 118 genes, of which 106 genes have distinctive expression during iron deprivation. Two common gene, *bfrA* and *fprA*, were regulated both in *M. tuberculosis* and *M. smegmatis*. Iron-depriving circumstances in the macrophage environment generate multiple stress conditions, which seem to trigger MrsI as part of an anticipatory response to iron limitation to help facilitate optimal survival of *M. tuberculosis* [112].

4.2. Protein sequestration

A ~300 nt long novel sRNA in *M. smegmatis* termed as Ms1 RNA [114] was identified as the most abundant non-rRNA transcript in stationary phase that sequesters core RNA polymerase(RNAP) lacking σ^A factors [115,116]. In contrast, 6S RNA, unlike Ms1 RNA, interacts with the RNAP holoenzyme [117–119], although Ms1 shares an analogous predicted secondary structure with 6S RNA-double stranded hairpin with a single stranded bubble in the center. Although Ms1 possesses two additional hairpins at the 5′ and 3′ ends, but the central bubble is essential for the interaction with RNAP core. A homologue (MTS2823) of Ms1 RNA exists in *M. tuberculosis* and predicted in other mycobacterial and actinobacterial species. Ms1expression level increases ~115-fold upon entry into the stationary phage [116] and is more stable in the stationary phase compared to exponential phase. Differential accumulation of Ms1 is controlled by an essential ribonuclease PNPase (3′–5′phosphorolytic activity). The build-up of Ms1 RNA in the stationary phase stems from the fact that elevation of its synthesis

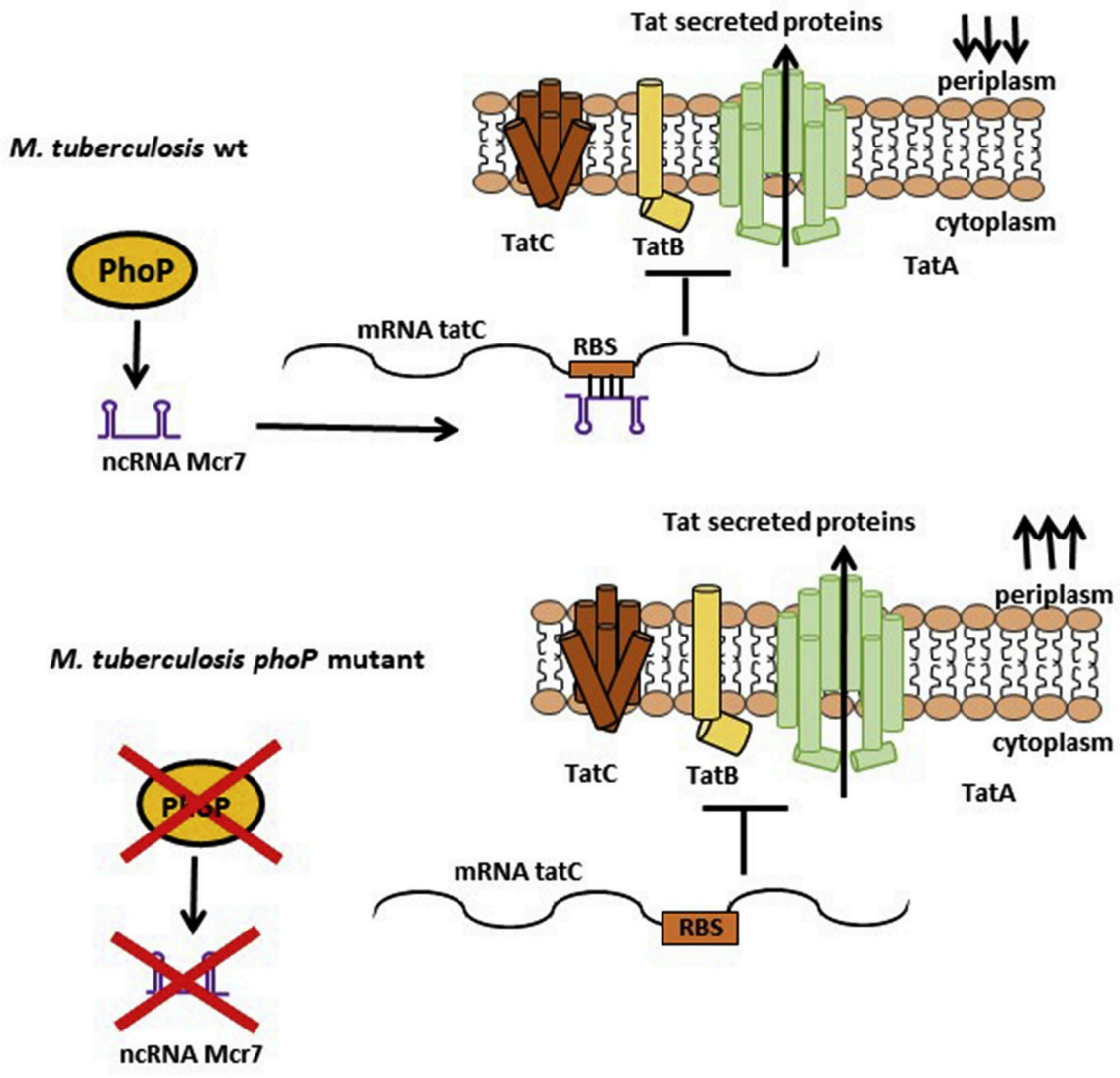

Fig. 3. PhoP-mediated regulation of *tatC* gene expression. *mcr7* transcription is upregulated by PhoP and subsequent antisense binding of Mcr7 to the 5'-end of *tatC* mRNA occludes its RBS for active translation. However, absence of Mcr7 in *ΔphoP* mutant cells does not repress TatC translation.

is integrated with reduced degradation. The activity of Ms1 RNA is regulated by its promoter P_{MS1} and *cis*-acting elements. Knockdown of *ms1* gene resulted in decreased level of mRNAs encoding β and β′ subunits of RNAP, which was also reflected in the protein level. Thus, during high transcriptional demand Δ*Ms1* strain provides smaller reservoir of RNAP, which leads to the inability of Δ*Ms1* strain to respond expeditiously to the changing environment during outgrowth from stationary phase [116] (Fig. 5). Ms1 homologue in *M. tuberculosis* MTS2823 is highly induced in chronic stages of lung infection, where MTS2823 may promote the pathogen survival in the belligerent environment. Ms1 could also be potentially connected to ethanol stress as under this stress condition Ms1 promoter activity increases [120]. The elevated level of Ms1 improves the cell fitness in the stationary phase where Δ*Ms1* strain is more vulnerable gamma radiation [116].

5. Conclusions and perspective

Pathogenic bacteria like *M. tuberculosis* are exposed to a wide array of stress conditions including oxidative stress, low pH, membrane stress, nutrient limitation etc. while infecting human host. Adaptation to this hostile environment is achieved by fine tuning their stress responsive genes at the transcriptional, posttranscriptional and translational level for survival [121–124]. The role of sRNA in stress response was not anticipated earlier, however, extensive investigations during last decade not only unveil the implication of sRNA in the adaptation to the stress environment, but also established their indispensable role in pathogenesis of different mycobacterial species. The discoveries of the diverse array of mycobacterial sRNAs shaded light on the mechanistic incidents behind how sRNAs exert their function. A systematic approach has been taken in this review to describe different strategies to identify mycobacterial sRNAs and also to comprehensively analyze how these sRNAs exert their regulatory role in different physiological processes.

Small RNAs have shown to act on their targets primarily through base pairing mechanism, the outcome of which is either the activation or the repression of their target gene expression [125]. However, it is still beyond our knowledge whether these sRNAs possess any distinctive structural feature to act either as an activator or a repressor of their target gene expression. To date only one protein binding sRNA (Ms1 RNA) has been discovered in mycobacterium [115,116], as the existence of the potential gene for 6S RNA is still controversial [115]. Ms1 is known to be induced in the stationary phase and increases the cell fitness to withstand stress conditions. However, how this sRNA interacts with RNAP core or whether it has any particular sequence motif for binding to RNAP core enzyme have yet to be investigated.

Except several housekeeping sRNAs like M1 RNA, tmRNA, 4.5S RNA

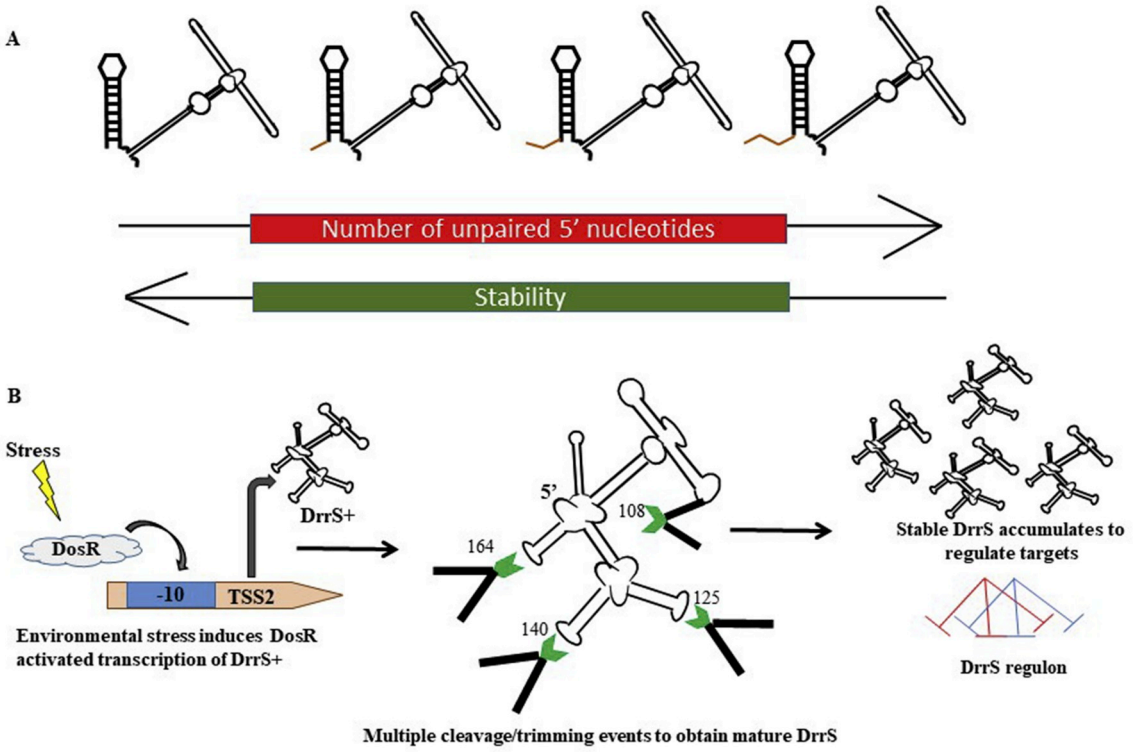

Fig. 4. (A) Stability of DrrS with varying length of unpaired residues at its 5'-end.Number of unpaired nucleotides at 5′ is inversely related to the stability of transcript.(B) DosR activation by hypoxia or NO stress leads to increased expression of DrrS+. Maturation of this transcript involves rapid processing at multiple places by ribonucleases. The resulting mature form DrrS is highly stable in the stationary phase of growth.

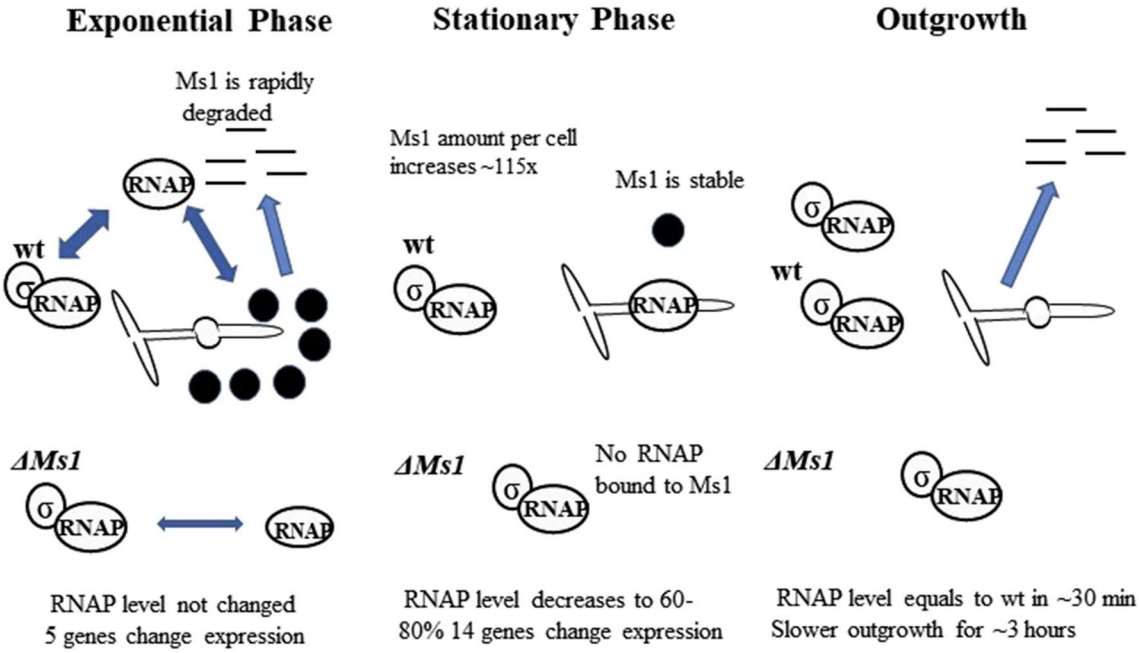

Fig. 5. The status of RNAP in wild type (wt) and Δ*Ms1* strains. Ms1 is unstable in the exponential phase and is degraded rapidly. The degradation is facilitated by PNPase. In *ΔMs1*, the level of RNAP remains unchanged as compared to wt. In the stationary phase, there is an interaction between RNAP and Ms1 in the wt strain, and a portion of RNAP is also sequestered by Ms1. No Ms1 is present in *ΔMs1*. Corresponding amount of RNAP is found to be missing when Ms1 is absent. This indicates that the absence of Ms1 is probably being compensated by the proportionate decreased fraction of the RNAP molecules. The group of RNAPs which are specific for transcription are similar in both wt and *ΔMs1* strains. During outgrowth, the difference in RNAP levels in both the strains is similar for approximately 30 min, and then the growth of *ΔMs1* is slowed down for about 3 h.

etc. in mycobacteria, nearly all sRNAs discovered to date are induced under diverse stress conditions in either of the stationary phase or the macrophage environment. Nearly 20% of total non-rRNA transcripts in exponentially growing *M. tuberculosis* emanate from IGR and represent sRNAs [6]. A substantial increase of this number to ~60% in stationary phase cells happens largely due to the accumulation of a highly abundant sRNA MTS2823, a homologue of Ms1 RNA in *M. smegmatis* [114]. The stockpiling of MTS2823 is even higher than rRNA amount in mice during chronic infection. Artificial overexpression of MTS2823 in the exponential phase of *M. tuberculosis* severely diminished the transcription of ~300 mycobacterial genes. All these events suggests that MTS2823 has a possible role during infection [70]. sRNAs in mycobacteria also can be upregulated under multiple stress conditions, which suggests their possible involvement in multiple regulatory network. MsrI in *M. tuberculosis* is highly induced in three different stresses. MsrI induced under iron starvation condition exhibited the colossal effects on mycobacterium transcriptome profile. Pre-exposure of *M. tuberculosis* to oxidative or any other stresses results in more rapid inhibition of *bfrA* expression under Fe-starvation condition. This indicates that *M. tuberculosis* receives an anticipatory signal upon discerning oxidative or membrane stress and remodels its gene expression for moving into macrophage environment with iron scarcity, priming MsrI to repress its target translation [112]. Studies on the well-characterized sRNAs in *E. coli* and *Salmonella* revealed that the function of an individual sRNA within a regulatory circuit differ substantially. A single sRNA can participate in multiple network depending on the interplay between sRNAs and targets [125]. On the contrary, a handful of sRNAs in *M. tuberculosis* have been validated to interact with their target and mode of their interaction are yet to be enlighten.

Research on identification of protein transcription factors or on small regulatory RNAs incriminated in pathogenesis has increased remarkably after the sequencing of *M. tuberculosis* genome. Analysis of genome wide mutagenesis discovered that nearly 200 protein encoding genes in *M. tuberculosis* genome are replaceable without affecting their growth but with attenuated virulence [126,127]. Multiple genetic loci have been denoted to be active in numerous physiological processes during infection e.g. adaptation to altering nutritional and stress conditions, restraining microbial defense against host immune system and production of macromolecules that balance the interaction with host cells [6]. sRNAs regulators have been detected by RNA-seq profiling to be associated with many of these loci [70]. Characterization of different recombinant phenotype of *M. tuberculosis* strains in terms of their adaptation and growth dynamics in presence of host immune cells was accomplished using a mouse model of infection. *M. tuberculosis* infection in human induces the generation of granulomatous lesions that provide heterogeneity of bacterial microenvironments [7]. The ability *M. tuberculosis* to tolerate the resulting truculent host environment and exploit that for bacterial multiplication and transmission will lead to successful pathogenesis. Upregulation of diverse sRNAs in retaliation to different stress conditions is possibly connected to multiple regulatory circuits which control cell survival. Thus, these sRNAs may serve as important biomarkers of the bacterial physiological status, supplying critical information for therapies [6].

Tuberculosis treatment is prolonged and a successful treatment requires medication for at least 6 months or more to remove residual bacterial population, which are resistant to antimicrobial drug. Thus, the salient research objectives in recent times aim to identify and synthesize a useful drug that will eliminate persistent mycobacterial population. Characteristics of resistant *M. tuberculosis* include the downregulation of a number of genes for their active growth. Profusion of sRNAs in *M. tuberculosis* cell and its interaction with the genes connected to pathogenesis implies that sRNAs orchestrate gene expression by playing a pivotal role in the posttranscriptional regulation for intracellular survival of this pathogenic bacteria. Phenotypically persistence feature of *M. tuberculosis* might probably be an outcome of sRNA-mediated modulation of several genes. Thus, the strategies to manipulate sRNA functions, which in consequence will alter the phenotypic properties of *M. tuberculosis*, will unfold a new dimension in therapeutic regimen for tuberculosis treatment.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Author contribution statement

TD designed the frame of writing. Both TD and ST studied the literature and wrote the paper.

Acknowledgement

We acknowledge Science and Engineering Research Board, Department of Science and Technology, Govt. of India for funding this research grant (ECR/2016/000178) to TD. ST would like to acknowledge the Indian Institute of Technology Delhi for fellowship.

References

[1] C.L. Cosma, D.R. Sherman, L. Ramakrishnan, The secret lives of the pathogenic Mycobacteria, Annu. Rev. Microbiol. 57 (2003) 641–676 https://doi.org/10.1146/annurev.micro.57.030502.091033.
[2] T. Jagielski, J. van Ingen, N. Rastogi, J. Dziadek, P.K. Mazur, J. Bielecki, Current methods in the molecular typing of Mycobacterium tuberculosis and other mycobacteria, BioMed Res. Int. (2014) 645802 https://doi.org/10.1155/2014/645802.
[3] J.V. Ingen, Diagnosis of nontuberculous mycobacterial infections, Semin. Respir. Crit. Care Med. 34 (1) (2013) 103–109 https://doi.org/10.1055/s-0033-1333569.
[4] J.D. Ernst, Macrophage receptors for Mycobacterium tuberculosis, Infect. Immun. 66 (1998) 1277–1281.
[5] A.M. Dannenberg Jr., Immuno-pathogenesis of pulmonary tuberculosis, Hosp. Pract. 28 (1993) 51–58 https://doi.org/10.1080/21548331.1993.11442738.
[6] K.B. Arnvig, D.B. Young, Noncoding RNA and its potential role in *Mycobacterium tuberculosis* pathogenesis, RNA Biol. 9 (4) (2012) 427–436 https://dx.doi.org/10.4161/rna.20105.
[7] L.G. Wayne, C.D. Sohaskey, Nonreplicating persistence of *Mycobacterium tuberculosis*, Annu. Rev. Microbiol. 55 (2001) 139–163 https://doi.org/10.1146/annurev.micro.55.1.139.
[8] C.E. Barry 3rd, H.I. Boshoff, V. Dartois, T. Dick, S. Ehrt, J. Flynn, et al., The spectrum of latent tuberculosis: rethinking the biology and intervention strategies, Nat. Rev. Microbiol. 7 (2009) 845–855 https://doi.org/10.1038/nrmicro2236.
[9] K. Haning, S.H. Cho, L.M. Contreras, Small RNAs in mycobacteria: an unfolding story, Front. Cellullar Infect. Microbiol. 4 (96) (2014) 1–11 https://dx.doi.org/10.3389/fcimb.2014.00096.
[10] J.L. Flynn, C.A. Scanga, K.E. Tanaka, J. Chan, Effects of aminoguanidine on latent murine tuberculosis, J. Immunol. 160 (1998) 1796–1803.
[11] J.L. Flynn, J. Chan, Tuberculosis: latency and reactivation, Infect. Immun. 69 (2001) 4195–4201 https://dx.doi.org/10.1128/IAI.69.7.4195-4201.2001.
[12] S.T. Cole, R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, et al., Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence, Nature 393 (1998) 537–544 https://doi.org/10.1038/31159.
[13] T. Garnier, K. Eiglmeier, J.C. Camus, N. Medina, H. Mansoor, M. Pryor, et al., The complete genome sequence of Mycobacterium bovis, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 7877–7882 https://doi.org/10.1073/pnas.1130426100.
[14] D.M. Scollard, Endothelial cells and the pathogenesis of lepromatous neuritis: insights from the armadillo model, Microb. Infect. 2 (2000) 1835–1843.
[15] S. Gottesman, G. Storz, Bacterial small RNA regulators: versatile roles and rapidly evolving variations, Cold Spring Harb. Perspect. Biol. 3 (2011) a003798 https://doi.org/10.1101/cshperspect.a003798.
[16] W. Wei, S. Zhang, J. Fleming, Z. Li, S. Fan, Y. Liu, W. Wang, Y. Liu, B. Ren, M. Wang, J. Jiao, Y. Chen, Y. Zhou, Y. Zhou, S. Gu, X. Zhang, L. Wan, T. Chen, L. Zhou, Y. Chen, X. Zhang, C. Li, H. Zhang, L. Bi, Mycobacterium tuberculosis type III-A CRISPR/Cas system crRNA and its maturation have atypical features, FASEB J. (2018), https://doi.org/10.1096/fj.201800557RR.
[17] Y. Zhang, J. Yang, G. Bai, Regulation of the CRISPR-associated genes by Rv2837c (CnpB) via an Orn-like activity in TB complex mycobacteria, J. Bacteriol. (2018), https://doi.org/10.1128/JB.00743-17.
[18] S. Schwenk, A. Moores, I. Nobeli, T.D. McHugh, K.B. Arnvig, Cell-wall synthesis and ribosome maturation are co-regulated by an RNA switch in Mycobacterium tuberculosis, Nucleic Acids Res. 46 (11) (2018) 5837–5849 https://doi.org/10.1093/nar/gky226.
[19] A.V. Sherwood, J.K. Frandsen, F.J. Grundy, T.M. Henkin, New tRNA contacts facilitate ligand binding in a *Mycobacterium smegmatis* T box riboswitch, Proc. Natl. Acad. Sci. U.S.A. 115 (15) (2018) 3894–3899 https://doi.org/10.1073/pnas.1721254115.
[20] S. Gottesman, The small RNA regulators of Escherichia coli: roles and mechanisms, Annu. Rev. Microbiol. 58 (2004) 273–301 https://doi.org/10.1146/annurev.micro.58.030603.123841.

[21] G. Gottesman, Micros for microbes: noncoding regulatory RNAs in bacteria, Trends Genet. 21 (2005) 399–404 https://doi.org/10.1016/j.tig.2005.05.008.
[22] S. Gottesman, G. Storz, Bacterial small RNA regulators: versatile roles and rapidly evolving variations, Cold Spring Harb. Perspect. Biol. 3 (2011) 1–16 https://doi.org/10.1101/cshperspect.a003798.
[23] K. Papenfort, J. Vogel, Multiple target regulation by small noncoding RNAs rewires gene expression at the post-transcriptional level, Res. Microbiol. 160 (2009) 278–287 https://doi.org/10.1016/j.resmic.2009.03.004.
[24] E.G.H. Wagner, S. Altuvia, P. Romby, Antisense RNAs in bacteria and their genetic elements, Adv. Genet. 46 (2002) 361–398.
[25] E.G. Wagner, F. Darfeuille, Small regulatory RNAs in bacteria, in: W. Nellen, C. Hamann (Eds.), Small RNAs: Analysis and Regulatory Functions, Springer Verlag, Berlin Heidelberg, Germany, 2006, pp. 1–29.
[26] E.G.H. Wagner, P. Romby, Small RNAs in bacteria and archaea: who they are, what they do, and how they do it, Adv. Genet. 90 (2015) 1–76 https://doi.org/10.1016/bs.adgen.2015.05.001.
[27] L.S. Water, G. Storz, Regulatory RNAs in bacteria, Cell 136 (2009) 615–628 https://doi.org/10.1016/j.cell.2009.01.043.
[28] G. Storz, J. Vogel, K.M. Wassarman, Regulation by small RNAs in bacteria: expanding frontiers, Mol. Cell 43 (2011) 880–891 https://doi.org/10.1016/j.molcel.2011.08.022.
[29] K.S. Frohlich, J. Vogel, Activation of gene expression by small RNA, Curr. Opin. Microbiol. 12 (2009) 674–682 https://doi.org/10.1016/j.mib.2009.09.009.
[30] M. Bobrovskyy, C.K. Vanderpool, Regulation of bacterial metabolism by small RNAs using diverse mechanisms, Annu. Rev. Genet. 47 (2013) 227–250 https://doi.org/10.1146/annurev-genet-111212-133445.
[31] T. Dutta, S. Srivastava, Small RNA-mediated regulation in bacteria: a growing palette of diverse mechanisms, Gene 656 (2018) 60–72 https://doi.org/10.1016/j.gene.2018.02.068.
[32] Y. Chao, J. Vogel, The role of Hfq in bacterial pathogens, Curr. Opin. Microbiol. 13 (2010) 24–33 https://doi.org/10.1016/j.mib.2010.01.001.
[33] K.M. Wasserman, 6S RNA: a small RNA regulator of transcription, Curr. Opin. Microbiol. 10 (2007) 156–163.
[34] P. Babitzke, T. Romeo, CsrB sRNA family: sequestration of RNA-binding regulatory proteins, Curr. Opin. Microbiol. 10 (2007) 156–163 https://doi.org/10.1016/j.mib.2007.03.007.
[35] C. Lucchetti-Miganeh, E. Burrowes, C. Baysse, G. Ermel, The post–transcriptional regulator CsrA plays a central role in the adaptation of bacterial pathogens to different stages of infection in animal hosts, Microbiology 154 (2008) 16–29 https://doi.org/10.1099/mic.0.2007/012286-0.
[36] K. Papenfort, J. Vogel, Regulatory RNA in bacterial pathogens, Cell Host Microbe 8 (2010) 116–127 https://doi.org/10.1016/j.chom.2010.06.008.
[37] C. Bohn, C. Rigoulay, P. Bouloc, No detectable effect of RNA-binding protein Hfq absence in *Staphylococcus aureus*, BMC Microbiol. 7 (2007) 10 https://doi.org/10.1186/1471-2180-7-10.
[38] Y. Liu, N. Wu, J. Dong, Y. Gao, X. Zhang, C. Mu, et al., Hfq is a global regulator that controls the pathogenicity of *Staphylococcus aureus*, PLoS One 5 (2010) 5 https://doi.org/10.1371/journal.pone.0013069.
[39] A. Smirnov, K.U. Forstner, E. Holmqvist, A. Otto, R. Gunster, D. Becher, R. Reinhardt, J. Vogel, Grad-seq guides the discovery of ProQ as a major small RNA-binding protein, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 11591–11596 https://doi.org/10.1073/pnas.1609981113.
[40] S.P. Pandey, B.K. Minesinger, J. Kumar, G.C. Walker, A highly conserved protein of unknown function in *Sinorhizobium meliloti* affects sRNA regulation similar to Hfq, Nucleic Acids Res. 39 (2011) 4691–4708 https://doi.org/10.1093/nar/gkr060.
[41] J. Vogel, B.F. Luici, Hfq and its constellation of RNA, Nat. Rev. Microbiol. 9 (2011) 578 https://doi.org/10.1038/nrmicro2615.
[42] J. Gripenland, S. Netterling, E. Loh, T. Tiensuu, A. Toledo-Arana, J. Johansson, RNAs: regulators of bacterial virulence, Nat. Rev. Microbiol. 8 (2010) 857–866 https://doi.org/10.1038/nrmicro2457.
[43] A. Toledo-Arana, O. Dussurget, G. Nikitas, N. Sesto, H. Guet-Revillet, D. Balestrino, et al., The Listeria transcriptional landscape from saprophytism to virulence, Nature 459 (2009) 950–956 https://doi.org/10.1038/nature08080.
[44] K.B. Arnvig, D.B. Young, Regulation of pathogen metabolism by small RNA, Drug Discov, Today Dis. Mech 7 (2010) 19–24.
[45] L. Feng, S.T. Rutherford, K. Papenfort, J.D. Bagert, J.C. van Kessel, D.A. Tirrell, N.S. Wingreen, B. Bassler, A Qrr noncoding RNA deploys four different regulatory mechanisms to optimize quorum-sensing dynamics, Cell 160 (2015) 228–240 https://doi.org/10.1016/j.cell.2014.11.051.
[46] S.L. Svenningsen, K.C. Tu, B.L. Bassler, Gene dosage compensation calibrates four regulatory RNAs to control *Vibrio cholerae* quorum sensing, EMBO J. 28 (2009) 429–439 https://doi.org/10.1038/emboj.2008.300.
[47] S. Chabelskaya, O. Gaillot, B. Felden, A *Staphylococcus aureus* small RNA is required for bacterial virulence and regulates the expression of an immune evasion molecule, PLoS Pathog. 6 (2010) 1000927 https://doi.org/10.1371/journal.ppat.1000927.
[48] B. Mann, T. Van Opijnen, J. Wang, C. Obert, Y.D. Wang, R. Carter, et al., Control of virulence by small RNAs in *Streptococcus pneumonia*, PLoS Pathog. 8 (2012) e1002788 https://doi.org/10.1371/journal.ppat.1002788.
[49] S.A. Roberts, J.R. Scott, RivR and the small RNA RivX: the missing links between the CovR regulatory cascade and the Mga regulon, Mol. Microbiol. 66 (2007) 1506–1522 https://doi.org/10.1111/j.1365-2958.2007.06015.x.
[50] Y. Wen, J. Feng, G. Sachs, *Helicobacter pylori* 5'-*ureB*-sRNA, a *cis*-encoded antisense small RNA, negatively regulates *ureAB* expression by transcription termination, J. Bacteriol. 195 (2012) 444–452 https://doi.org/10.1128/JB.01022-12.
[51] S.M. Gaida, M.A. Al-Hinai, D.C. Indurthi, S.A. Nicolaou, E.T. Papoutsakis, Synthetic tolerance: three noncoding small RNAs, DsrA, ArcZ and RprA, acting supra-additively against acid stress, Nucleic Acids Res. 41 (2013) 8726–8737 https://doi.org/10.1093/nar/gkt651.
[52] T. Soper, P. Mandin, N. Majdalani, S. Gottesman, S. Woodson, Positive regulation by small RNAs and the role of Hfq, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 9602–9607 https://dx.doi.org/10.1073/pnas.1004435107.
[53] J.R. Mellin, P. Cossart, The non-coding RNA world of the bacterial pathogen *Listeria monocytogenes*, RNA Biol. 9 (2012) 372–378 https://doi.org/10.4161/rna.19235.
[54] I. Caldelari, Y. Chao, P. Romby, J. Vogel, RNA-mediated regulation in pathogenic bacteria, Cold Spring Harb. Perspect. Med. 3 (2013) a010298 https://doi.org/10.1101/cshperspect.a010298.
[55] A. Tomasini, P. François, B.P. Howden, P. Fechter, P. Romby, I. Caldelari, The importance of regulatory RNAs in *Staphylococcus aureus*, Infect. Genet. Evol. 21 (2014) 616–626 https://doi.org/10.1016/j.meegid.2013.11.016.
[56] H. Gong, G.P. Vu, Y. Bai, E. Chan, R. Wu, E. Yang, et al., A *Salmonella* small non-coding RNA facilitates bacterial invasion and intracellular replication by modulating the expression of virulence factors, PLoS Pathog. 7 (2011) 1002120 https://doi.org/10.1371/journal.ppat.1002120.
[57] T. Mizuno, M.Y. Chou, M. Inouye, A unique mechanism regulating gene expression: translational inhibition by a complementary RNA transcript (micRNA), Proc. Natl. Acad. Sci. U. S. A. 81 (7) (1984) 1966–1970.
[58] K.M. Wassarman, F. Repoila, C. Rosenow, G. Storz, S. Gottesman, Identification of novel small RNAs using comparative genomics and microarrays, Genes Dev. 15 (2001) 1637–1651 https://doi.org/10.1101/gad.901001.
[59] L. Argaman, R. Hershberg, J. Vogel, G. Bejerano, E.G.H. Wagner, H. Margalit, S. Altuvia, Novel small RNA-encoding genes in the intergenic regions of Escherichia coli, Curr. Biol. 11 (2001) 941–950.
[60] E. Rivas, R.J. Klein, T.A. Jones, S.R. Eddy, Computational identification of noncoding RNAs in E. coli by comparative genomics, Curr. Biol. 11 (2001) 1369–1373.
[61] S. Hen, E.A. Lesnik, T.A. Hall, R. Sampath, R.H. Griffey, D.J. Ecker, L.B. Blyn, A bioinformatics based approach to discover small RNA genes in the Escherichia coli genome, Biosystems 65 (2002) 157–177 https://doi.org/10.1016/S0303-2647(02)00013-8.
[62] A. Sittka, S. Lucchini, K. Papenfort, C.M. Sharma, K. Rolle, T.T. Binnewies, J.C.T. Hinton, J. Vogel, Deep sequencing analysis of small noncoding RNA and mRNA targets of the global post-transcriptional regulator, Hfq. PLoS Genet. 4 (2008) e1000163 https://doi.org/10.1371/journal.pgen.1000163.
[63] C.M. Sharma, J. Vogel, Experimental approaches for the discovery and characterization of regulatory small RNAs, Curr. Opin. Microbiol. 12 (2009) 536–546 https://doi.org/10.1016/j.mib.2009.07.006.
[64] S. Melamed, A. Peer, R. Faigenbaum-Romm, Y.E. Gatt, N. Reiss, A. Bar, Y. Altuvia, L. Argaman, H. Margalit, Global mapping of small RNA-target interactions in bacteria, Mol. Cell 63 (2016) 884–897 https://doi.org/10.1016/j.molcel.2016.07.026.
[65] K.B. Arnvig, D.B. Young, Identification of small RNAs in Mycobacterum tuberculosis, Mol. Microbiol. 73 (3) (2009) 397–408 https://doi.org/10.1111/j.1365-2958.2009.06777.x.
[66] J. Panek, J. Bobek, K. Mikulik, M. Basler, J. Vohradsky, Biocomputational prediction of small non-coding RNAs in *Streptomyces*, BMC Genomics 9 (2008) 217 https://doi.org/10.1186/1471-2164-9-217.
[67] C. Bohn, C. Rigoulay, P. Bouloc, No detectable effect of RNA-binding protein Hfq absence in *Staphylococcus aureus*, BMC Microbiol. 7 (2007) 10 https://doi.org/10.1186/1471-2180-7-10.
[68] E. Geisinger, R.P. Adhikari, R. Jin, H.F. Ross, R.P. Novick, Inhibition of rot translation by RNAIII, a key feature of *agr* function, Mol. Microbiol. 61 (2006) 1038–1048 https://doi.org/10.1111/j.1365-2958.2006.05292.x.
[69] J.M. Dichiara, L.M. Contreras-Martinez, J. Livny, D. Smith, K.A. Mcdonough, M. Belfort, Multiple sRNAs identified in Mycobacterium bovis BCG are also expressed in Mycobacterium tuberculosis and Mycobacterium smegmatis, Nucleic Acids Res. 38 (12) (2010) 4067–4078 https://dx.doi.org/10.1093/nar/gkq101.
[70] K.B. Arnvig, I. Coma, N.R. Thomson, J. Houghton, H.I. Boshoff, N.J. Croucher, G. Rose, T.T. Perkins, J. Parkhill, G. Dougan, D.B. Young, Sequence-based analysis uncovers an abundance of non-coding RNA in the total transcriptome of Mycobacterium tuberculosis, Plos Pathogn 7 (2011) e1002342 https://doi.org/10.1371/journal.ppat.1002342.
[71] C.H. Tsai, C. Baranowski, J. Livny, K.A. Mcdonough, J.T. Wade, L.M. Contreras, Identification of novel sRNAs in Mycobacterial species, PLoS One 8 (2013) e79411 https://doi.org/10.1371/journal.pone.0079411.
[72] M. Wang, J. Fleming, Z. Li, H. Zhang, Y. Xue, M. Chen, Z. Zhang, X.E. Zhang, L. Bi, An automated approach for global identification of sRNA-encoding regions in RNA-seq data from *Mycobacterium tuberculosis*, Acta Biochim. Biophys. Sin. 48 (6) (2016) 544–553 https://doi.org/10.1093/abbs/gmw037.
[73] T. Akama, K. Suzuki, K. Tanigawa, A. Kawashima, H. Wu, M. Sue, A. Yoshihara, Y. Ishido, N. Ishii, Whole genome expression analysis of Mycobacterium leprae and its clinical application, Jpn. J. Infect. Dis. 63 (2010) 387–392.
[74] S.K. Li, P.K.S. NG, H. Qin, J.K.Y. Lau, J.P.Y. Lau, S.K.W. Tsui, T.F. Chan, T.C.K. Lau, Identification of small RNAs in Mycobacterium smegmatis using heterologous Hfq, RNA 19 (2013) 74–84 https://doi.org/10.1261/rna.034116.112.
[75] S. Schwenk, K.B. Arnvig, Regulatory RNA in Mycobacterium tuberculosis, back to basics, Pathogens Disease 76 (4) (2018) 1–12 https://doi.org/10.1093/femspd/fty035.
[76] T.B. Updegrove, S.A. Shabalina, G. Storz, How do base-pairing small RNAs evolve? FEMS Microbiol. Rev. 39 (2015) 379–391 https://doi.org/10.1093/femsre/fuv014.
[77] H. Otaka, H. Ishikawa, T. Morita, H. Aiba, PolyU tail of rho-independent terminator of bacterial small RNAs is essential for Hfq action, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 13059–13064 https://doi.org/10.1073/pnas.1107050108.
[78] E. Sauer, O. Weichenrieder, Structural basis of RNA 3′-end recognition by Hfq, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 13065–13070 https://doi.org/10.1073/pnas.1103420108.

[79] G. Desnoyer, B. Bouchard Marie-Pier, E. Masse, New insights into small RNA dependent translation regulation in prokaryotes, Trends Genet. 29 (2012) 92–98 https://doi.org/10.1016/j.tig.2012.10.004.
[80] M. Boudvillain, N. Figueroa-Bossi, A role for rho-dependent polarity in gene regulation by a noncoding small RNA, Genes Dev. 26 (2012) 1864–1873 https://dx.doi.org/10.1101/gad.195412.112.
[81] Y. Chao, L. Li, D. Girodat, K.U. Forstner, N. Said, C. Corcoran, M. Smiga, K. Papenfort, R. Reinhardt, H.J. Wieden, B. Luisi, J. Vogel, In vivo cleavage map illuminates the central role of RNase E in coding and non-coding RNA pathway, Mol. Cell 65 (2017) 39–51 https://doi.org/10.1016/j.molcel.2016.11.002.
[82] E. Massé, F.E. Escorcia, S. Gottesman, Coupled degradation of a small regulatory RNA and its mRNA targets in Escherichia coli, Genes Dev. 17 (2003) 2374–2383 https://doi.org/10.1101/gad.1127103.
[83] E. Sonnleitner, U. Bläsi, Regulation of hfq by the RNA CrcZ in pseudomonasaeruginosa carbon catabolite repression, PLoS Genet. 10 (2014) e1004440 https://doi.org/10.1371/journal.pgen.1004440.
[84] M. Bobrovsky, C.K. Vanderpool, The small RNA SgrS: roles in metabolism and pathogenesis of enteric bacteria, Front. Cellular Infec. Microbiol. 4 (2014) 1–8 https://doi.org/10.3389/fcimb.2014.00061.
[85] K. Papenfort, C.K. Vanderpool, Target activation of regulatory RNAs in bacteria, FEMS Microbiol. Rev. 39 (2015) 362–378 https://doi.org/10.1093/femsre/fuv016.
[86] J.A. Opdyke, J.G. Kang, G. Storz, GadY, a small RNA regulator of acid response genes in Escherichia coli, J. Bacteriol. 186 (2004) 6698–6705 https://doi.org/10.1128/JB.186.20.6698-6705.2004.
[87] J.A. Opdyke, E.M. Fozo, M.R. Hemm, G. Storz, RNase III participates in GadY dependent cleavage of the gadX-gadW mRNA, J. Mol. Biol. 406 (2011) 29–43 https://doi.org/10.1016/j.jmb.2010.12.009.
[88] P. Babitzke, T. Romeo, CsrB sRNA family: sequestration of RNA binding regulatory proteins, Curr. Opin. Microbiol. 10 (2007) 156–163 https://doi.org/10.1016/j.mib.2007.03.007.
[89] C.S. Baker, I. Morozov, K. Suzuki, T. Romeo, P. Babitzke, CsrA regulates glycogen biosynthesis by preventing translation of glgC in Escherichia coli, Mol. Microbiol. 44 (2002) 1599–1610 https://doi.org/10.1046/j.1365-2958.2002.02982.x.
[90] K. Suzuki, P. Babitzke, S.R. Kushner, T. Romeo, Identification of a novel regulatory protein (CsrD) that targets the global regulatory RNAs CsrB and CsrC for degradation by RNase E, Genes Dev. 20 (2006) 2605–2617 https://dx.doi.org/10.1101/gad.1461606.
[91] D. Schnappinger, et al., Transcriptional adaptation of Mycobacterium tuberculosis within macrophages: insights into the phagosomal environment, J. Exp. Med. 198 (2003) 693–704 2003 https://doi.org/10.1084/jem.20030846.
[92] G.R. Stewart, et al., The heat shock response of Mycobacterium tuberculosis: linking gene expression, immunology and pathogenesis, Comp. Funct. Genom. 3 (2002) 348–351 https://dx.doi.org/10.1002/cfg.183.
[93] Y. Zhou, J. Xie, The roles of Pathogen small RNAs, J. Cell. Physiol. 226 (2010) 968–973.
[94] C.M. Sassetti, D.H. Boyd, E.J. Rubin, Genes required for mycobacterial growth defined by high density mutagenesis, Mol. Microbiol. 48 (2003) 77–84 https://doi.org/10.1046/j.1365-2958.2003.03425.x.
[95] I. Matsunaga, A. Bhatt, D.C. Young, T.Y. Cheng, S.J. Eyles, G.S. Besra, et al., *Mycobacterium tuberculosis pks12* produces a novel polyketide presented by CD1c to T cells, J. Exp. Med. 200 (2004) 1559–1569 https://doi.org/10.1084/jem.20041429.
[96] D. Schnappinger, S. Ehrt, M.I. Voskuil, Y. Liu, J.A. Mangan, I.M. Monahan, et al., Transcriptional adaptation of *Mycobacterium tuberculosis* within macrophages: insights into the phagosomal environment, J. Exp. Med. 198 (2003) 693–704 2003 https://doi.org/10.1084/jem.20030846.
[97] J. Houghton, T. Cortes, O. Schubert, G. Rose, A. Rodgers, M.D.X. Croix, A. Aebersold, D.B. Young, K.B. Arnvig, A small RNA encoded in the Rv2660c locus of Mycobacterium tuberculosis is induced during starvation and infection, PLoS One 8 (12) (2013) e80047.
[98] S. Sturgill-Koszycki, P.H. Schlesinger, P. Chakraborty, P.L. Haddix, H.L. Collins, A.K. Fok, R.D. Allen, S.L. Gluck, J. Heuser, D.G. Russell, Lack of acidification in Mycobacterial phagosomes produced by exclusion of the vesicular proton-ATPase, Science 263 (1994) 678–681 https://doi.org/10.1126/science.8303277.
[99] I.M. Monohan, J. Bettes, D.K. Banerjee, P.D. Butcher, Differential expression of mycobacterial proteins following phagocytosis by macrophages, Microbiology 147 (2001) 459–471 https://doi.org/10.1099/00221287-147-2-459.
[100] R.J. Hobson, A.J. McBride, K.E. Kempsell, J.W. Dale, Use of an arrayed promoter -probe library for the identification of macrophage-regulated genes in Mycobacterium tuberculosis, Microbiology 148 (2002) 1571–1579 https://dx.doi.org/10.1099/00221287-148-5-1571.
[101] M.A. Gazdik, G. Bai, Y. Wu, K.A. McDonough, Rv1675c (cmr) regulates intramacrophage and cAMP-induced gene expression in Mycobacterium tuberculosis-complex mycobacteria, Mol. Microbiol. 71 (2009) 434–448 https://doi.org/10.1111/j.1365-2958.2008.06541.x.
[102] K.B. Arnvig, B. Gopal, K.G. Papavinasasundaram, R.A. Cox, M.J. Colston, The mechanism of upstream activation in the rrnB operon of Mycobacteriumsmegmatis is different from the Escherichia coli paradigm, Microbiology 151 (2005) 467–473 https://dx.doi.org/10.1099/mic.0.27597-0.
[103] L. Solans, J. Gonzalo-Asensio, C. Sala, et al., The PhoP-dependent ncRNAMcr7 modulates the TAT secretion system in Mycobacterium tuberculosis, PLoS Pathog. 10 (2014) e1004183 https://doi.org/10.1371/journal.ppat.1004183.
[104] S.B. Walters, E. Dubnau, I. Kolesnikova, F. Laval, M. Daffe, I. Smith, The Mycobacterium tuberculosis PhoPR two-component system regulates genes essential for virulence and complex lipid biosynthesis, Mol. Microbiol. 60 (2) (2006) 312–330 https://doi.org/10.1111/j.1365-2958.2006.05102.x.
[105] E. Perez, S. Samper, Y. Bordas, C. Guilhot, B. Gicquel, C. Martin, An essential role for phoP in Mycobacterium tuberculosis virulence, Mol. Microbiol. 41 (1) (2001) 179–187 https://doi.org/10.1046/j.1365-2958.2001.02500.x.
[106] J.S. Lee, R. Krause, J. Schreiber, H.J. Mollenkopf, J. Kowall, R. Stein, B.Y. Jeon, J.Y. Kwak, M.K. Song, J.P. Patron, S. Jorg, K. Roh, S.N. Cho, S.H.E. Kaufmann, Mutation in the transcriptional regulator PhoP contributes to avirulence of Mycobacterium tuberculosis H37Ra strain, Cell Host Microbe 3 (2) (2008) 97–103 https://doi.org/10.1016/j.chom.2008.01.002.
[107] R. Brosch, S.V. Gordon, M. Marmiesse, P. Brodin, C. Buchrieser, K. Eiglmeier, T. Garnier, C. Gutierrez, G. Hewinson, K. Kremer, L.M. Parsons, A.S. Pym, S. Samper, D. van Soolingen, S.T. Cole, A new evolutionary scenario for the Mycobacterium tuberculosis complex, Proc. Natl. Acad. Sci. U. S. A. 99 (6) (2002) 3684–3689 https://doi.org/10.1073/pnas.052548299.
[108] B.S. Joanis, C. Demangel, M. Jackson, P. Brodin, L. Marsollier, H. Boshoff, S.T. Cole, Inactivation of Rv2525c, a substrate of the twin arginine translocation (Tat) system of Mycobacterium tuberculosis, increases beta-lactam susceptibility and virulence, J. Bacteriol. 188 (2006) 6669–6679 https://doi.org/10.1128/JB.00631-06.
[109] T. Palmer, B.C. Berk, The twin-arginine translocation (Tat) protein export pathway, Nat. Rev. Microbiol. 10 (7) (2012) 483–496 https://doi.org/10.1038/nrmicro2814.
[110] A. Moores, A.B. Riesco, S. Schwenk, K.B. Arnvig, Expression, maturation and turnover of DrrS, an unusually stable, DosR regulated small RNA in Mycobacterium tuberculosis, PLoS One 12 (3) (2017) e0174079 https://doi.org/10.1371/journal.pone.0174079.
[111] S. Chauhan, D. Sharma, A. Singh, A. Surolia, J.S. Tyagi, Comprehensive insights into Mycobacterium tuberculosis DevR (DosR) regulon activation switch, Nucleic Acids Res. 39 (17) (2011) 7400–7414 https://doi.org/10.1093/nar/gkr375.
[112] E.R. Gerrick, T. Barbier, M.R. Chase, R. Xu, J. Francois, V.H. Lin, M.J. Szucs, J.M. Rock, R. Ahmed, B. Tjaden, J. Livny, S.M. Fortune, Small RNA profiling in Mycobacterium tuberculosis identifies MsrI as necessary for an anticipatory iron sparing response, Proc. Natl. Acad. Sci. U.S.A. 115 (25) (2018) 6464–6469 https://doi.org/10.1073/pnas.1718003115.
[113] J.M. Rock, F.F. Hopkins, A. Chavez, M. Diallo, M.R. Chase, E.R. Gerrick, J.R. Pritchard, G.M. Church, E.J. Rubin, C.M. Sassetti, D. Schnappinger, S.M. Fortune, Programmable transcriptional repression in mycobacteria using an orthogonal CRISPR interference platform, Nat. Microbiol. 2 (2017) 16274 https://doi.org/10.1038/nmicrobiol.2016.274.
[114] J. Panek, L. Krasny, J. Bobek, E. Jezkova, J. Korelusova, J. Vohradsky, The suboptimal structures find the optimal RNAs: homology search for bacterial noncoding RNAs using suboptimal RNA structures, Nucleic Acids Res. 39 (2011) 3418–3426 https://doi.org/10.1093/nar/gkq1186.
[115] J. Hnilicova, J.J. Mtejckova, M. Sikova, J. Pospisil, P. halada, J. Panek, L. Krasny, Ms1, a novel sRNA interacting with RNA polymerase core in mycobacteria, Nucleic Acids Res. 42 (18) (2014) 11763–11776 https://doi.org/10.1093/nar/gku793.
[116] M. Sikova, M. Janouskova, O. Ramaniuk, P. Palenikova, J. Pospisil, P. Bartl, A. Suder, P. Pajer, P. Kubickova, O. Pavlis, M. Hradilova, D. Vitovska, H. Sanderova, M. Prevorovsky, J. Hnilicova, L. Krasny, Ms1 RNA increases the amount of RNA polymerase in Mycobacterium smegmatis, Mol. Microbiol. (2018) (ahead of print), https://doi.org/10.1111/mmi.14159.
[117] K.M. Wassarman, G. Storz, 6S RNA regulates E. coli RNA polymerase activity, Cell 101 (2000) 613–623 https://doi.org/10.1016/S0092-8674(00)80873-9.
[118] A.T. Cavanagh, K.M. Wasserman, 6S RNA, a global regulator of transcription in Escherichia coli, Bacillus subtilis and beyond, Annu. Rev. Microbiol. 68 (2014) 45–60 https://doi.org/10.1146/annurev-micro-092611-150135.
[119] B. Steuten, S. Schnelder, R. Wagner, 6S RNA: recent answers - future questions, Mol. Microbiol. 91 (2014) 641–648 https://doi.org/10.1111/mmi.12484.
[120] H. Cao, D. Wei, Y. Yang, Y. Shang, G. Li, Y. Zhou, Q. Ma, Y. Xu, Systems-level understanding of ethanol-induced stresses and adaptation in E. coli, Sci. Rep. 7 (2017) 44150.
[121] U.E. Schaible, S.H. Kaufmann, Iron and microbial infection, Nat. Rev. Microbiol. 2 (2004) 946–953 https://doi.org/10.1038/nrmicro1046.
[122] C.L. Stallings, M.S. Glickman, Is Mycobacterium tuberculosis stressed out? A criticalassessment of the genetic evidence, Microb. Infect. 12 (2010) 1091–1101 https://doi.org/10.1016/j.micinf.2010.07.014.
[123] G.E. Purdy, M. Niederweis, D.G. Russell, Decreased outer membrane permeability protects mycobacteria from killing by ubiquitin-derived peptides, Mol. Microbiol. 73 (2009) 844–857 https://doi.org/10.1111/j.1365-2958.2009.06801.x.
[124] P.T. Liu, S. Stenger, D.H. Tang, R.L. Modlin, Cutting edge: vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin, J. Immunol. 179 (2007) 2060–2063 https://doi.org/10.4049/jimmunol.179.4.2060.
[125] C.L. Beisel, G. Storz, Base pairing small RNAs and their roles in global regulatory networks, FEMS Microbiol. Rev. 34 (2010) 866–882.
[126] C.M. Sassetti, E.J. Rubin, Genetic requirements for mycobacterial survival during infection, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 12989–12994 https://doi.org/10.1073/pnas.2134250100.
[127] C.M. Sassetti, D.H. Boyd, E.J. Rubin, Genes required for mycobacterial growth defined by high density mutagenesis, Mol. Microbiol. 48 (2003) 77–84 https://doi.org/10.1046/j.1365-2958.2003.03425.x.