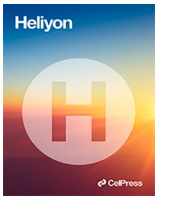
Heliyon 9 (2023) e22611

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Mycobacterium tuberculosis complex molecular networks and their regulation: Implications of strain heterogeneity on epigenetic diversity and transcriptome regulation

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A R T I C L E I N F O

Keywords:
Tuberculosis
MTBC
Heterogeneity
Epigenetics
sRNA
Transcriptome
Whole genome sequence
SMRT sequencing

A B S T R A C T

Tuberculosis has been a public health crisis since the 1900, which has caused the highest mortalities due to a single bacterial infection worldwide, that was recently further complicated by the Coronavirus disease 2019 pandemic. The causative agent of Tuberculosis, *Mycobacterium tuberculosis*, belongs to a genetically well-characterized family of strains known as the *Mycobacterium tuberculosis* complex, which has complicated progress made towards eradicating Tuberculosis due to pathogen-specific phenotypic differences in the members of this complex. *Mycobacterium tuberculosis* complex strains are genetically diverse human- and animal-adapted pathogens belonging to 7 lineages (Indo-Oceanic, East-Asian, East-African Indian, Euro-American, *M. africanum* West Africa 1, *M. africanum* West Africa 2 and Ethopia), respectively and the recently identified Lineage 8 and *M. africanum* Lineage 9. Genomic studies have revealed that *Mycobacterium tuberculosis* complex members are ~99 % similar, however, due to selective pressure and adaptation to human host, they are prone to mutations that have resulted in development of drug resistance and phenotypic heterogeneity that impact strain virulence. Furthermore, members of the *Mycobacterium tuberculosis* complex have preferred geographic locations and possess unique phenotypic characteristics that is linked to their pathogenicity. Due to the recent advances in development next generation sequencing platforms, several studies have revealed epigenetic changes in genomic regions combined with "unique" gene regulatory mechanisms through non-coding RNAs that are responsible for strain-specific behaviour on *in vitro* and *in vivo* infection models. The current review provides up to date epigenetic patterns, gene regulation through non-coding RNAs, together with implications of these mechanisms in down-stream proteome and metabolome, which may be responsible for "unique" responses to infection by members of the *Mycobacterium tuberculosis* complex. Understanding lineage-specific molecular mechanisms during infection may provide novel drug targets and disease control measures towards World Health organization END-TB strategy.

1. Introduction

Despite increasing interventions and funding directed towards eradicating the tuberculous bacilli, 1,6 million deaths and 10,6 new

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https://doi.org/10.1016/j.heliyon.2023.e22611
Received 14 January 2023; Received in revised form 13 November 2023; Accepted 15 November 2023
Available online 19 November 2023

million cases were estimated by World health organization (WHO) in 2022, which was further complicated by the emergence of a global pandemic, COVID19. There was an 18 % decline in the actual reported cases of tuberculosis (TB) between 2019 and 2020, which spiked very rapidly in the emergence of the global COVID19 pandemic between 2020 and 2022 [1]. Due to these high incidences, TB is reported as one of the 21st-century public health emergencies. Furthermore, the high estimated prevalence that is reported in TB is largely due to the emergence and persistence of multi-, extremely- and total drug-resistant (MDR, XDR, and TDR) strains, respectively [2,3], delay in diagnosis of the disease and the low protection by the current BCG TB vaccine beyond childhood [4] as well as uncharacterized molecular mechanisms that confer strain-specific virulence traits. A group of closely related species comprises a family, of which all cause TB in humans and animals, belongs to the *Mycobacterium tuberculosis* complex (MTBC). MTBC consists of genetically similar strains such as *M. tuberculosis*, which is the main infectious agent responsible for TB in humans. This complex also includes *M. bovis*, *M. canetti,* and *M. africanum* that are also prominent in clinical practices, as well as animal adapted *M. mungi*, *M. orygis*, *M. suricattae*, *M. microti*, *M. caprae*, and *M. pinnipedi* (rarities) [5]. *M. tuberculosis*, being most renowned of the nine lineages [6–8], is acknowledged as infecting one-third of the world's population. MTBC has been established to consist of two distinct clades. The first clade solely adapted to human hosts is a modern lineage that is characterized by the TbD1 deletion, which include genotype families that belong to lineage 2 (East-Asian), 3 (East-African Indian) and 4 (Euro-American). The ancient clade harboring the TbD1 genomic region include the human- and animal-adapted genotypes belonging to lineage 1 (Indo-Oceanic), 5 and 6 (*M. africanum* West Africa 1 and West Africa 2, respectively) and 7 (Ethiopia) [6], respectively and the recently identified Lineage 8 [7] and *M. africanum* Lineage 9 [8]. High throughput analyses have confirmed the diversity and pathogenic differences in human- and animal-adapted MTBC lineages, which is a consequence of adaption to different hosts [9] and geographic locations [6,10] leading to virulence determinants that are strain-specific [11].

Genetic diversity and strain heterogeneity play a major role in the determination of the consequence of *M. tuberculosis* infection as well as several factors including virulence, resistance, and immunogenicity [12]. Previously, *M. tuberculosis in vitro* and *in vivo* pathogenesis studies have broadly focused on the *M. tuberculosis* H37Rv and Erdman laboratory strains [13]. However, meaningful genetic differences between these laboratory strain and various other clinical isolates are apparent, imparting varying ranges of virulence [14–16]. Pathogenesis studies [17,18], profiling the host responses to varying genotypes of *M. tuberculosis*, observed that the Beijing and LAM4 genotypes, belonging to East-Asian lineage 2 and Euro-American lineage 4, respectively, are implicated in increased bacterial multiplication followed by early pneumonia and cytotoxic responses. Furthermore, reduced immune response to MTBC modern lineages may be linked to rapid disease progression and transmission, which confers selective advantage for these strains compared to ancient lineages [19,20]. Thus, it is apparent that genetically diverse clinical strains of *M. tuberculosis* belonging to different lineages induce specific responses that can influence the outcome of the disease and possibly contribute to their virulence and transmission.

There has been an increased interest towards better understanding of MTBC molecular networks that govern their virulence, drug resistance, host response and transmissibility by exploiting next generation sequencing (NGS) technologies [21,22]. In the late 2000s, several studies identified *cis*- and *trans*-encoded non-coding RNAs [23–25] in several species of *Mycobacterium*. Early pathogen-specific *M. tuberculosis* transcriptome studies [26–28] further indicated regulation of stress-response pathways and unique gene regulation profiles associated with clinical isolates of MTBC, implying strain-specific transcriptional regulatory mechanisms. Furthermore, NGS technologies have recently identified many other regulatory non-coding sRNAs [29–34], and MTBC-specific methylation patterns [35–41] that govern the molecular networks of these notorious pathogens. Therefore, the current review provides an update on all the known non-coding regulatory RNAs and their associated transcripts, together with the methylome data of *M. tuberculosis*. Furthermore, MTBC-specific molecular regulators and networks that have lineage-specific clinical implication will also be discussed.

1.1. Literature search methodology

Google search, Google scholar and PubMed were used to select the reviewed literature using the following keywords: WHO Tuberculosis report, *Mycobacterium tuberculosis* complex (MTBC), MTBC lineages, MTBC genetic Heterogeneity, Host response to MTBC, *M. tuberculosis* Whole Genome Sequence, MTBC Epigenetics and Methylome, *M. tuberculosis* SMRT Sequencing, *Mycobacterium* noncoding RNAs, MTBC Transcriptome diversity. Literature search was not filtered using publication year as many discoveries in *Mycobacterium* gene regulation were made in the late 90s to early 2000s.

1.2. Consequence of MTBC diversity and their influence on host response

Genomic interrogation of MTBC strains has revealed that they exhibit ~99 % nucleotide homology [39,42], with conserved lineage-specific synonymous and nonsynonymous single nucleotide polymorphisms (SNPs) [43]. However, several studies have shown significant differences between clinically relevant strains of MTBC in terms of their phenotypic characteristics such as growth rate, host response, transmissibility, cell wall lipids and other unique virulence traits [10,42,44–47]. Early *in vitro* and *in vivo* studies [48–51] indicated that clinical strains of *M. tuberculosis* exhibit differential growth rate, followed by diverse cytokine and chemokine responses. Manca et al. [50] indicated that the Beijing strain, HN878, of the East-Asian lineage was hypervirulent as demonstrated by unusually early death of infected immuno-competent mice, compared to infection with other clinical isolates of the same strain family (NHN5, HN60), CDC1551 and the laboratory H37Rv of the Euro-American lineage. Strains of the same lineage or family sub-lineages can induce variable *in vitro* and *in vivo* survival patterns and cytokines responses based on their genetic characterization. This was shown by modern Beijing sub-lineages possessing intact RD150 and RD142 regions, that induced very low levels of pro-inflammatory cytokines compared to ancient Beijing strains, which might hypothetically explain their predominance in Taiwan [20]. Low predominance of

M. africanum strains among the human TB causing genotypes might be linked to their slow growth rate and consistently low cytokine response during infection on the *in vivo* mice model compared to the Beijing and the laboratory H37Rv of the East-Asian and Euro-American lineages, respectively [52]. Furthermore, modern strains of the Euro-American lineage show a higher rate of replication, associated with a significant production of proinflammatory cytokines and activation of autophagy, compared to the ancient strains of Indo-Oceanic and *M. africanum* West Africa 1, respectively [53].

Previous studies have shown that *M. tuberculosis* strains that belongs to the Beijing family of the East-Asian lineage are globally prevalent [54–59] and hypervirulent on *in vitro* [60] and *in vivo* [50] infection models with unique cell wall lipid profiles [61]. Previously, we have shown that the Beijing strain induced low cytokine response [62] and increased enrichment of cholesterol biosynthesis pathway [63] compared to other clinical strains of the Euro-American lineage. Furthermore, we previously showed unique molecular signatures [64] and host post transcriptional alternative splicing events [65] in response to each clinical strain (Beijing, F11, F28, F15/LAM4/KZN, Unique) of *M. tuberculosis*, which strongly suggest that MTBC lineages possess unique virulence traits that are responsible for specific response during *in vitro* and *in vivo* infection models. Recently, it has been shown that the infecting MTBC induce distinct cytokine response in whole blood stimulated by strains of different lineages. Overall, strains of Euro-American lineage induced low production of pro-inflammatory cytokines compared to other lineages. In particular, significantly low IL-2 and IFN-γ cytokine response was observed for Euro-American lineage compared to East-African Indian; while the modern lineages induced low IL17 compared to ancestral lineages [66]. Moreover, modern MTBC lineages generally induce low macrophage cytokine response [19], which might contribute to their successful transmissibility in the 21st century. Collectively, findings from *in vitro*, *in vivo* and patient response to clinical strains of MTBC lineages indicate varying response to infecting strain, suggesting strain-specific virulence traits exhibited by these strains. In summary, the modern strains of MTBC such as the Beijing genotype of East-Asian lineage exhibit high transmissibility and low cytokine response, while the second globally dominant Euro-American lineage exhibit low growth rate and low cytokine response. Ancient lineages seem to exhibit low virulence and generally high cytokine response combined with low global prevalence (Fig. 1); without neglecting strain-specific phenotypic differences of the same genotype family and lineage that might induce differing responses in pathogenesis studies. Despite overwhelming research and data generated in recent years in this area, full *in vitro* and *in vivo* pathogenesis studies that include representative of each lineage as proposed by Borrell, Trauner [67] still remain to be fully characterized, with the inclusion of the recently identified Lineage 8 [7] and Lineage 9 [8] strains.

Differences in behaviour of the MTBC strains on *in vitro* and *in vivo* infection models as well as their distribution and transmission rates among the population alludes to complex molecular mechanisms that are "uniquely" regulated among these clinically relevant pathogens. Strides have been made in understanding how members of the MTBC regulate their molecular pathways as described in

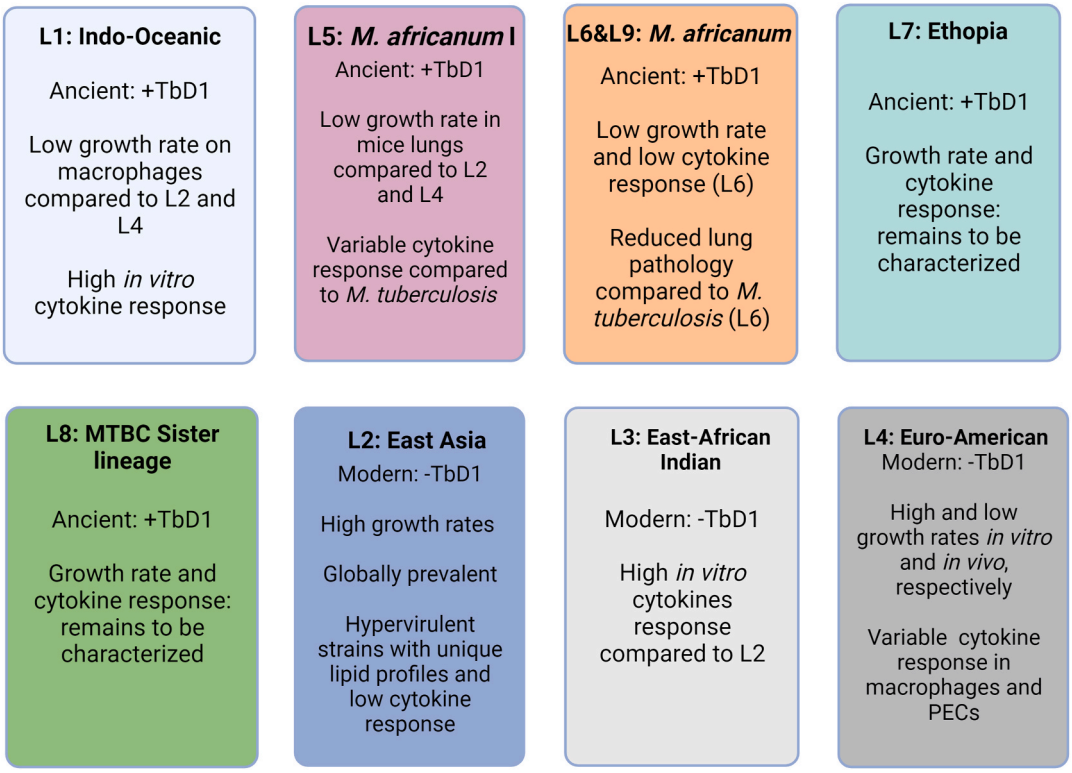

Fig. 1. MTBC members and their phenotypic differences associated with *in vitro* and *in vivo* "unique" responses in pathogenesis studies. L: Lineage; PEC: Pulmonary epithelial cells. Ancient strains induce low immune activation while modern strains with high transmissibility induce higher cytokine response, with few exceptions.

detail in the following sections.

1.3. Methylation in Mycobacterium tuberculosis complex: Motifs with clinical relevance

DNA methylation is an epigenetic mechanism that plays a role in gene expression, and evidence has shown that *M. tuberculosis* genomes contain N6-methyladenine (m6A) and 5-methylcytosine (m5C) methylation mechanisms [68]. Using Single-molecule real-time (SMRT) sequencing, characterization identified three DNA methyltransferases (MTase), *mamA*, *mamB*, and *hsdM* that control the m6A methylation [69]. Two recent studies have shown evidence that specific MTase mutation results in altered or loss of MTase activity resulting in strain-specific methylation patterns [68]. Lineage-specific methylation patterns have also been reported in MTBC strains [70]. Strain-specific methylation profiles may play a role in *M. tuberculosis* evolution and cause the observed gene function and phenotypic heterogeneity [69,71].

Changes in methylation patterns/motifs in MTBC genomes is essential in regulating bacterial response to a changing environment, resulting in differentiation of gene expression and function. A study investigating the relationship between methylation status and gene expression concluded that the Euro-American lineage (Lineage 4) CTCCAG methylation motif had the greatest impact on gene expression of *ompA*, *Rv1371*, *scpB*, *moaC3* and *Rv3324A* transcripts [69]. Within Euro American lineage, 38 genes showed differential expression due to the CTCCAG motif, while only 2 and 4 genes were affected by GATN$_4$RTAC and CACGCAG, respectively [69]. Strains with the *mamA* G152S mutation exhibited 28 uniquely non-methylated genes, which were mostly downregulated [69]. The *mamA* MTase methylates the CTCCAG motif, which is located in metabolic regulator genes, suggesting that methylation plays a role in these functions. (Table 1). An example of a downregulated gene within Euro American lineage is *Rv0348*, which is associated with regulatory proteins and metabolic pathways of *M. tuberculosis* strains [69].

A recent study has attributed the loss of *mamA* MTase function in East African lineage to the A809C (E270A) point mutation [71] (Table 1). Loss of *mamA* MTase function has been shown to affect survival in hypoxic conditions and gene expression due to the role methylation plays in transcription [72]. Beijing strains of the East African lineage are known for their higher tendency to cause active disease and increasing accumulation of resistance genes [71]. Modern strains, such as members of East African Indian and East-Asian lineages, have been observed to have a large number of lineage-specific recombination, which could explain their evolutionary adaptation and tendency to cause active disease [71]. Additionally, a greater number of genomic rearrangements of genes associated with cell wall components such as PPE, PE-PGRS and ESAT-6 in both ancient and modern lineages at different rates provides evidence of lineage-specific tendency to transmit disease [71].

Similarly to the loss of *mamA* MTase function, studies showed that a novel missense mutation (S253L) resulted in a loss of *mamB* MTase in some isolates within the Indo-Oceanic lineage [71]. The *mamB* MTase methylates the CACGCAG motif in Indo-Oceanic lineage and was shown to enrich fatty acid and polyketide synthesis pathways [68]. The consequence of the S253L mutation is not fully known but could contribute to the increased transmission of the East African Indian 6 family in Brazil [73].

In the study conducted by Chu et al. [74], the methylomes of TDR, XDR and susceptible strains were sequenced using SMRT sequencing. The methylome profile of the resistant clinical strains showed no significant differences with the susceptible strains. After treatment with anti-TB drugs, the frequency of methylated sequences among the detected motifs were nearly 100 % in all drug-resistance and drug-susceptible isolate, suggesting that drug stress had no impact on genome methylation [74]. Transduction was used to knockout the *hsdM* gene from the XDR strain 11,826 (11826Δ*hsdM*) to define role of HsdM in pathogenesis and drug resistance. The loss of HsdM resulted in complete demethylation of the CTAYN4ATC motif and a significantly increase or decrease in the regulation of drug target and transporter transcripts [74]. A significant increase in the levels of *katG* and *embB* mRNA was observed is the 11826Δ*hsdM* mutant compared to its parent strain [74], suggesting that DNA methylation regulates drug resistance at a transcriptional level. Additionally, a few HsdM regulated genes were shown to be associated with cellular respiration and could interfere with nicotinamide adenine dinucleotide (NAD/NADH) ratios. Previous studies showed that bacterial isoniazid (INH) susceptibility is affected by NAD/NADH [75], thus suggesting that HsdM affects drug susceptibility by interfering with redox related pathways, which remains to be investigated further in future studies using transcriptomics and proteomics tools.

M. tuberculosis strains have been shown to have remarkably complex sugar and lipid complexes within the cell wall [76], which affects pathogenicity in disease causing MTBC strains [77]. Mycolic acids are an essential component of the cell envelope, which consists of high lipid content and forms the external mycomembrane that encapsulates the *M. tuberculosis* [76]. A variety of unique MTases are utilized in the biosynthesis of mycolic acids and are crucial for survivability, antibiotic resistance and virulence [77]. A

Table 1
Methylation patterns of different MTBC lineages and the consequences of heterogeneity.

Methylation motif	Function	Strain/Lineage	Consequence of MTBC diversity
CTCCAG	Metabolic regulatory proteins	Lineage 4	Down regulation of gene expression
CTCCAG	Role in transcription	Lineage 2	Decreased gene expression and hypoxia survival
CACGCAG	Enrichment of fatty acid and Polyketide synthesis pathways	Lineage 1	Increased virulence
GATNNNNRTAC	Enrichment of cell and plasma membrane associated genes	Lineage 4	Decreased antibiotic susceptibility
GTAYN4ATC	Respiration and drug resistance-related genes	Strain 11,826	Altered drug resistances and susceptibility
CTCGAG	Cell wall biosynthesis and modification	*M. smegmatis* strain mc^{2}155	Acquisition of resistance to anti-mycobacterial factors

recent study investigated Rv1523 MTase to determine its role in mycolic acid and cell wall lipid production [76]. The study utilized Search Tool for the Retrieval of Interacting Genes (STRING) analysis and showed that Rv1523 could play a role in the modification and biosynthesis of cell wall components [76]. It was shown that Rv1523 is a vital MTase in the production in cell wall fatty acids within the FAS-II pathway [76]. The expression *Rv1523* in *M. smegmatis* results in host immunity modulation by decreasing TNF-α proinflammatory production and increasing IL10 anti-inflammatory production. This suggests that Rv1523-mediation cell wall alterations affect the pathophysiology and acquisition of resistance to anti-mycobacterial factors in *Mycobacterium* [76].

Previous studies hypothesized that DNA methylation plays a role in transcription, which suggests that differences in gene transcription profiles should occur if there is differentiation in methylation. Based on recent studies, DNA methylation has shown the potential to contribute to strain and lineage-specific differences in clinically relevant MTBC strains. The presence of mutation within methylation motifs and MTase genes has been shown to alter the methylation profile between strains. This suggests that MTBC strain heterogeneity correlates with an alteration in methylome profiles resulting in phenotypic differences between genetically similar strains. Future studies should integrate transcriptomic data to determine which methylation mechanisms are associated with virulence and antibiotic resistance. This could uncover novel mechanisms to be exploited in order to develop new treatment methods.

1.4. Transcriptome diversity and regulation: A step closer to understanding MTBC strain phenotypes

M. tuberculosis possess the ability to transition between latent and active states in response to various stressors and environments, making it a successful pathogen. Alterations in its global transcriptional profile highlights these adaptations [78]. Furthermore, lineage-specific SNPs was shown to alter the transcriptome of Indo-Oceanic and East-Asian lineages, resulting in differential gene expression with unique transcriptional regulatory changes [43]. Transcriptome is tightly regulated by the non-coding sRNAs in bacterial species [79], including *M. tuberculosis* [23,24]. Small noncoding RNAs (sRNAs) make up the largest group of regulatory RNAs. They consist of 50–500 nt long transcripts that calibrate bacterial gene expression in response to stress, in order to undergo rapid adaptation [80]. They have a vital function in the interactions between the host and the pathogen, as well as a role in the regulation of the interactions between the pathogen and its environment. *M. tuberculosis* utilizes the regulatory function of sRNAs for their rapid adaptation to a shift in the environmental surrounding [81].

The sRNAs possess the ability to adjust the result of gene expression even after the initial process has occurred. This is achieved by one of two ways; promotion or inhibition of the synthesis of bacterial proteins that play a major role in impeding the success of the host's defence strategies [82]. Various processes such as transcription, translation, mRNA stability, and DNA maintenance or silencing are regulated by sRNAs [83]. Their primary function is in pathogenesis and response to stress, with additional roles in quorum sensing, transportation, metabolism, etc. [82]. There are two groups of sRNAs; (i) those that are transcribed from intergenic regions with multiple targets (*trans*-encoded) and (ii) those transcribed in the antisense direction to the target mRNA (*cis*-encoded), as shown in Fig. 2 [23]. Both *cis*- and *trans*-encoded sRNAs consist of their own promoters and terminators, as they are encoded by individual genes. *Cis*- and *trans*-encoded sRNAs prevent translation of their target mRNA through inhibition of mRNA binding to the ribosome-binding

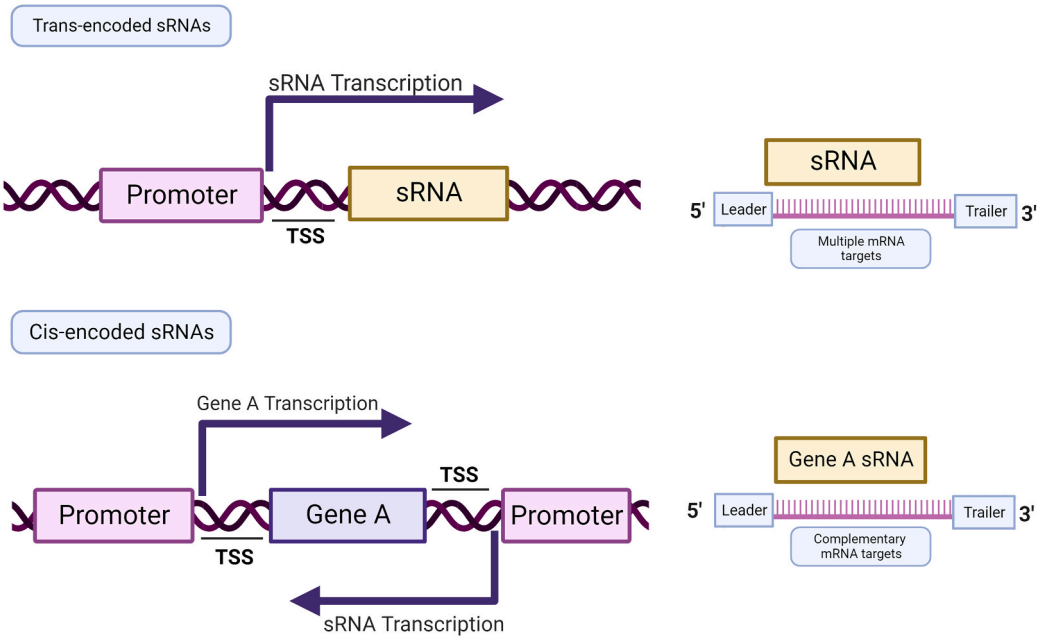

Fig. 2. Illustration of bacterial *trans*- and *cis*-encoded sRNA transcription and mRNA regulation. *Trans*-encoded sRNAs are independent of the target gene and under regulation of their own promoters that are activated upon receiving environmental stimulation with multiple mRNA targets. *Cis*-encoded sRNAs are encoded in the opposite strand of their target mRNA using their own promoters. TSS: Transcription start site.

site (RBS), resulting in the failure to produce the protein [81].

Understanding of *M. tuberculosis* molecular networks and their regulation have been an ongoing mission since the discovery of TB. In the mid to late 2000s, Arnvig and Young [24] were among the first authors to describe the presence of sRNAs in *M. tuberculosis* through their identification of four (ASdes, ASpks, AS1726, AS1890) *cis*-encoded sRNAs that are antisense to *desA1*, *pks12*, *Rv1726* and *Rv1890c* transcripts; respectively, and five (B11, B55, C8, F6 and G2) *trans*-encoded sRNAs. These authors subjected *M. tuberculosis* to oxidative stress and observed increased regulation of B11, B55, F6 (also known as ncRv10243, MTS194, MTB000051) and ASpks sRNAs. Among the identified sRNAs, F6 was detected during acid-stress [24] and has been recently shown to be produced during macrophage infection and nutrient deficiency [84]. F6 is conserved over a broad spectrum of mycobacteria, with the 5′ terminus exhibiting the most conservation. It is located between *fadA2* and *fadE5* genes, which encode acetyl coenzyme A (acetyl-CoA) transferase and acyl-CoA hydrogenase, respectively, and remains highly conserved, suggesting that F6 may play a role in lipid metabolism, which is essential for mycobacterial intracellular survival [84].

The advances made in sequencing technologies and development of bacterial transcriptome regulation databases such as BS_finder [33], RNAPredict2 [85], RNomics [86] and RNAz [87] in the past decades has allowed scientist to identify and characterize more *M. tuberculosis* sRNAs (including ncRv11147Ac, ncRv2395, ncRv11534A, MTS2823, MTS1338, Mcr11, Mcr7, MrsI and MTS2048), while thousands of other sRNA predictions have been made through *in silico* [83] tools that remains to be confirmed and functionally characterized as excellently reviewed by Haning et al. [81] and Ostrik et al. [88]. MTS2823, is found downstream of *Rv3661* and is highly conserved in mycobacteria and is known to be involved in protein binding rather than mRNA regulation. MTS2823 over-expression in *M. tuberculosis* reduced growth rate followed by up- and down-regulation of several transcripts [89] and is highly elevated in mice infection model [90]. On exposure to stress, during the passage from the logarithmic to stationary phase, as well as during infection, Mcr11, MTS1338 and MTS2823 sRNAs amass [23,89]. Differences in external stimulus has been associated with transcriptome changes which can be linked to accumulation of specific sRNAs as regulatory tools of these changes. Iron deficiency in *M. tuberculosis* increases MrsI (also known as MTB000142, ncRv11846) sRNA [91] while ncRv12659 (also identified as MTS2048) accumulate during infection of mice models [92]. Furthermore, Mcr7 is among sRNA that is associated with mycobacterial pathogenesis as it is regulated by a two component PhoPR signalling system, which is essential for virulence of mycobacteria [93]. An extensive review providing detailed functions of *M. tuberculosis* sRNAs, structure and their regulation has been provided by Ostrik et al. [88].

Clinical strains of MTBC have shown a very high degree of conservation, making it a successful pathogen, which has translated to detection of conserved gene regulators including sRNAs [94]. Despite this high degree of genetic conservation in MTBC strains, differential pathogen transcriptome changes during *in vitro* macrophages infection [26], cholesterol rich media [95] and *in vivo* infection [90], suggesting "unique' gene regulation by sRNAs has been reported. Recently, Alvarez-Eraso et al. [96] showed that silencing Mcr11 sRNAs in two Colombian clinical strains (UT127 and UT205) and a laboratory H37Rv strain induce distinct growth phenotype. The H37Rv and UT127 strains showed delayed growth patterns, while growth was inhibited in the UT205 strain, which also exhibited a clumping phenotype. Furthermore, upon transcriptome interrogation during Mcr11 silencing, the laboratory H37Rv and UT127 strain showed similar gene regulation patterns, which vastly differed from the UT205 strain. The "unique" UT205 transcriptional signatures may be associated with reduction in macrophage cell death compared to the wild type of the same strain [96], which may provide better insight into strain-specific *M. tuberculosis* virulent-specific sRNAs. Understanding the mechanisms of virulent-specific sRNAs in clinically relevant MTBC strains will be a step closer to designing novel tools to control the growth and virulence of this "notorious" pathogen with a strain-specific perspective of the MTBC lineages.

2. Conclusion and future perspective

Despite many strides made in TB detection, treatment and management, this disease remains as one of the challenging infectious diseases of the 21st century, especially in resource constrained countries, which has been further complicated by the recent COVID19 pandemic. Clinically relevant MTBC strain are ~99 % similar genetically, however, they exhibit differences in growth phenotypes and on *in vitro* and *in vivo* models. These differences may be due to specific methylome and gene regulatory patterns that are unique to the lineages of this complex and even strain-specific patterns within the same strain family. Consequences of genetic heterogeneity can be observed in transmission dynamics in different geographic locations as well as on *in vitro* and *in vivo* models. Furthermore, recent epigenetic studies have associated non-genetic patterns with drug resistance, increased virulence as well as gene regulation within the members of MTBC.

Recent advances made on sRNAs and other non-coding RNAs as central gene regulators with a wide variety of tasks within a cell's regulatory circuits have been reported in MTBC. Recent studies have provided evidence, pertaining the mechanisms of these sRNAs and their impact on pathogenesis as well as their potential as diagnostic markers [97]. Thousands of *M. tuberculosis* sRNAs have been identified since their discovery in 2009 through the use of computational tools and a limited number have been confirmed and functionally characterized. Thus, there are still many gaps in understanding how each of the ~4000 genes of *M. tuberculosis* are regulated and their roles in virulence and pathogenesis through uncovering the mechanisms of their regulators. Only recently, a strain-specific perspective on sRNA gene regulation and phenotype has been characterized [96] on *M. tuberculosis* clinical strains oof Columbian origin and this is an area that still needs to be further investigated within the members of MTBC. In conclusion, there is still a lot more to uncover in order to understand MTBC clinically relevant strains and the consequence of their genetic heterogeneity in gene regulatory mechanisms through non-coding RNAs and epigenetic modulation during different stages of growth and during infection of different models.

Data availability statement

Data will be made available upon request from the corresponding author (Mvubu, NE).

Ethics declaration

Review and/or approval by an ethics committee was not needed for this study because the compiled data was obtained through already published manuscripts.

CRediT authorship contribution statement

Nontobeko Eunice Mvubu: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Kieran Jacoby:** Writing – original draft, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors will like to declare no conflict of interest.

Acknowledgements

The authors wish to acknowledge Miss Arlia Salig for literature search that was used for the Transcriptome diversity and regulation: A step closer to understanding MTBC strain phenotypes section. This research was funded by the Foundation Botnar under the EDCTP2 programme (TMA2020CDF-3167), supported by the European Union.

Abbreviations

TB	Tuberculosis
MTBC	Mycobacterium tuberculosis complex
COVID19	Coronavirus disease 2019
MDR	multi drug-resistant
XDR	extremely drug-resistant
TDR	total drug-resistant
NGS	next generation sequencing
sRNA	small RNA
SNP	single nucleotide polymorphism
RD	Region of Difference
PEC	Pulmonary epithelial cells
SMRT	Single-molecule real-time
MTase	methyltransferase
m6A	N6-methyladenine
m5C	5-methylcytosine
INH	isoniazid

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