



# Important role of DNA methylation hints at significant potential in tuberculosis

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## Abstract

Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis* (Mtb) infection, has persisted as a major global public health threat for millennia. Until now, TB continues to challenge efforts aimed at controlling it, with drug resistance and latent infections being the two main factors hindering treatment efficacy. The scientific community is still striving to understand the underlying mechanisms behind Mtb's drug resistance and latent infection. DNA methylation, a critical epigenetic modification occurring throughout an individual's growth and development, has gained attention following advances in high-throughput sequencing technologies. Researchers have observed abnormal DNA methylation patterns in the host genome during Mtb infection. Given the escalating issue of drug-resistant Mtb, delving into the role of DNA methylation in TB's development is crucial. This review article explores DNA methylation's significance in human growth, development and disease, and its role in regulating Mtb's evolution and infection processes. Additionally, it discusses potential applications of DNA methylation research in tuberculosis.

**Keywords** DNA methylation · Tuberculosis · DNMT · Infection and immunity

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## Introduction

Most cells in an organism carry the same genetic information, but not all cell types simultaneously activate all genes, a phenomenon closely linked to epigenetic regulation. Epigenetic regulation refers to heritable changes in gene function without altering the DNA sequence, ultimately causing phenotypic changes in an individual (Waddington 2012). This regulation includes DNA methylation, histone acetylation, and RNA methylation. DNA methylation, a common DNA modification, is crucial in controlling various biological processes in organisms. The advent and widespread use of high-throughput sequencing technologies in recent years have drawn increasing attention to the role of DNA methylation in regulating human diseases, shedding light on the mechanisms behind disease occurrence. In several studies, it was observed that the development of many diseases is accompanied, to varying degrees, by abnormal DNA methylation within the host genome. Intervention targeting this aberrant methylation can be highly beneficial in treating certain diseases. For instance, 5-aza-2'-deoxycytidine (decitabine, DAC) has gained widespread use as an FDA-approved DNA methyltransferase inhibitor in the treatment of diseases such as acute myeloid leukemia (AML)

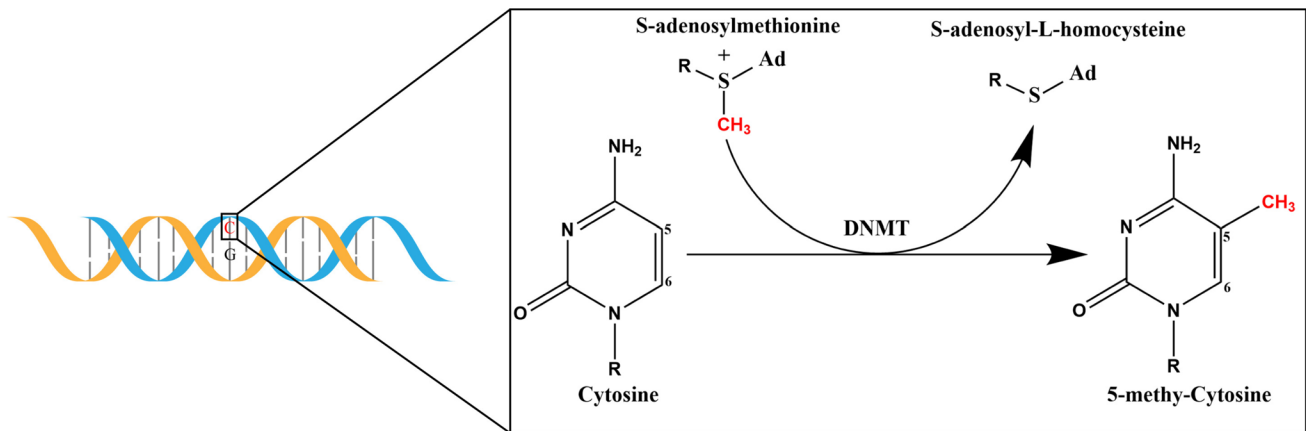
and myelodysplastic syndromes (MDS) (Crujisen et al. 2014). In addition to cancer, numerous pathogens can also manipulate host immunity through DNA methylation modifications, thereby enhancing their survival within the host. Host-related genes associated with processes like cell cycle progression, senescence, inflammation, and immunity are critical targets for pathogens involved in this regulatory process (Jaenisch and Bird 2003). Pero et al. 2011 discovered that *Helicobacter pylori* (Hp) induces abnormal methylation of the host COX-2 gene, leading to the development of associated intestinal diseases (Pero et al. 2011). The capacity of pathogen infections to introduce novel methylation patterns into the host's DNA suggests the need for a deeper comprehension of pathogen-organism interactions from an epigenetic perspective. Furthermore, it underscores that inhibiting abnormal methylation for therapeutic purposes also applies to infection immunity.

*Mycobacterium tuberculosis* (Mtb) is a pathogenic bacterium that spreads through respiratory droplets, leading to tuberculosis (TB) infection. The World Health Organization (WHO) classified TB as a major global pandemic in 1993. According to the newly released survey, TB will cause about 1.3 million deaths globally in 2022, second only to COVID-19. Although the number of deaths caused by TB in 2022 is lower than the 1.4 million in 2021 and 2020, there will be approximately 7.5 million new cases of TB globally in 2022 (WHO 2023). This is the highest number counted in a year since WHO began testing for TB globally in 2019. In addition, about a quarter of the world's population is infected with Mtb, with an additional 10 percent of them suffering from lifelong active tuberculosis. (Kinsella et al. 2021). The tuberculin skin test and the  $\gamma$ -interferon (IFN- $\gamma$ ) release assay (IGRA) are the primary methods employed to detect Mtb infection, relying on a positive T-cell response to Mtb antigens. Nevertheless, some individuals test seropositive but do not develop active TB. These cases are categorized as latent TB infection (LTBI). While latently infected individuals may not exhibit overt clinical symptoms, they face a significant risk of developing active TB and potentially contributing to the TB epidemic in their respective regions, especially when their immune systems are compromised. Recognizing the vast scale of this issue, the World Health Organization has prioritized the treatment of latent TB-infected individuals as a pivotal component of its strategy to eradicate TB. Currently widely used LTBI regimens include rifapentine plus isoniazid once weekly for 3 months, rifampicin once daily for 4 months, or isoniazid plus rifampicin once daily for 3 months. Isoniazid once daily for 6 or 9 months is also an alternative (Shah and Dorman 2021). In addition to latent TB infections, the emergence of drug-resistant Mtb strains has further undermined the effectiveness of traditional Mtb detection and treatment methods. The types of TB caused by infections with drug-resistant mutant Mtb strains include

multi-drug-resistant (MDR), pre-extensively drug-resistant (Pre-XDR, and extensively drug-resistant (XDR) (Mazurek et al. 2005). The reference treatment regimens provided by the WHO for these two characteristics of Mtb are generally extended treatment cycles or multi-drug combinations. However, developing host-directed therapies (HDT) to enhance host immunity is a must if TB is to be completely eradicated. And this requires a deeper understanding of the Mtb infection process. Currently, two primary methods for diagnosing TB are in vivo or in vitro assessments of the body's response to Mtb antigens, detected using the tuberculin skin test (TST) or interferon-gamma release assays (IGRAs). However, recent research indicates that differences in IGRA responses are more valuable for assessing TB development in children, whereas these differences hold less significance in adults (Gupta et al. 2020). Furthermore, some individuals continue to test negative for TST or IGRAs despite exposure to areas with a high prevalence of TB or environments with elevated levels of Mtb. For example, a study by Catherine M. Stein revealed that approximately 10% of South African miners remained TST-negative after many years of work, even though the environments they worked in likely had some of the world's highest Mtb concentrations (Stein et al. 2018). Simultaneously, Mtb infection can introduce DNA methylation patterns into host chromosomes, affecting biological processes such as host gene expression, DNA replication, and the immune response to promote its survival. It has been established that Mtb infection introduces novel DNA methylation patterns into the host genome, and several differentially expressed methylation sites in latent versus active TB have been reported (Du et al. 2022). Given its prevalence as an epigenetic regulation mechanism in both eukaryotic and prokaryotic organisms, the role of DNA methylation in immunity to Mtb infection presents promising research avenues. This review explores DNA methylation modifications and their roles in the human body, focusing on their significance during Mtb infection. Additionally, we discuss how DNA methylation influences host-related immune modulation during Mtb infection and the potential applications of DNA methylation in TB research.

## DNA methylation

DNA methylation is commonly defined as the process wherein the methyl group ( $-CH_3$ ) from S-adenosylmethionine (SAM) is transferred to the fifth carbon atom (C5) of cytosine, resulting in the formation of C5-methylcytosine, often referred to as 5-methylcytosine (5mC). This biochemical reaction is catalyzed by DNA methyltransferase (DNMT) (Moore et al. 2013) (Fig. 1). Apart from the C5 position of cytosine, which serves as the primary site for methylation, the sixth nitrogen atom of adenine can also undergo



**Fig. 1** Mechanism of DNA methylation. In the presence of DNA methyltransferase (DNMT), the methyl group (red colour) of S-adenosylmethionine (SAM) is transferred to the fifth carbon atom of a

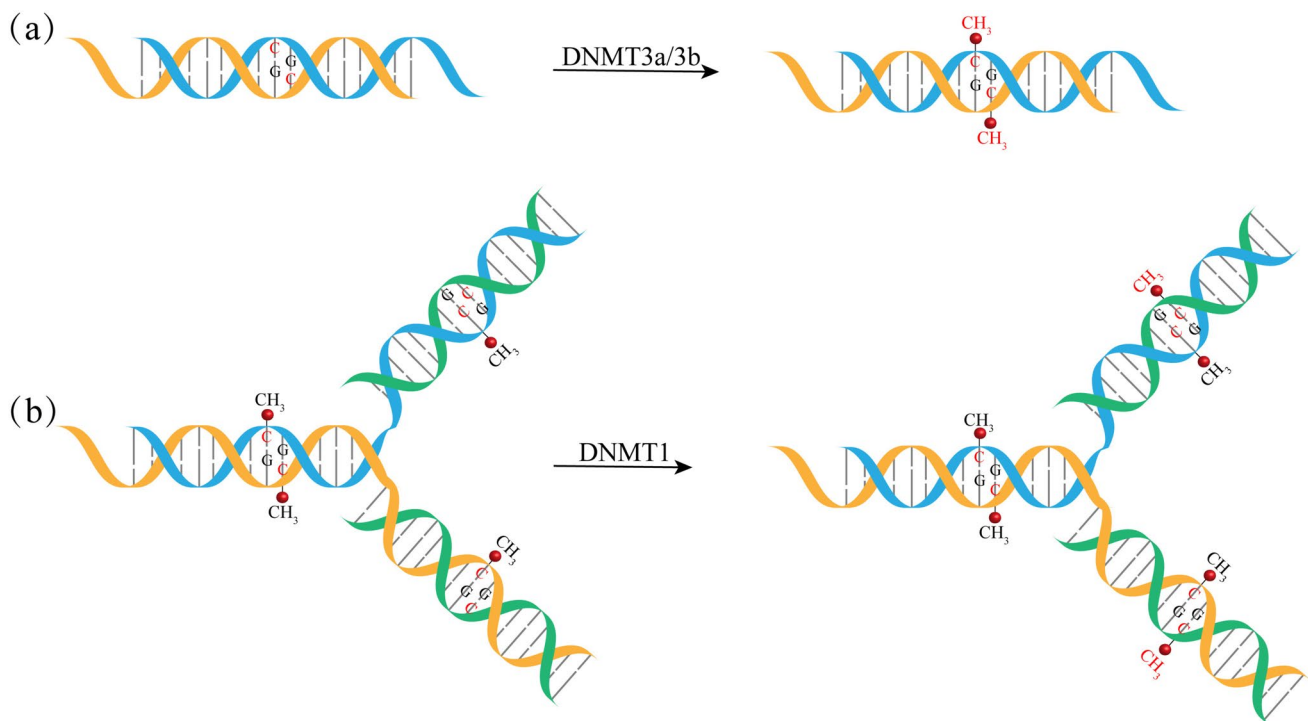
cytosine of CpG to produce s-adenosyl- L-homocysteine (SAH) and 5-methylcytosine (5-mC) (color figure online)

methylation, forming N6-methyladenine (6 mA). Additionally, the fourth nitrogen atom of cytosine can be methylated, yielding N4-methylcytosine (4mC) as a modification product (Ponger and Li 2005). While the first two DNA methylation modifications are present in prokaryotic and eukaryotic genomes, 4mC is currently exclusively identified in specific prokaryotic genomes. The predominant form of DNA methylation within eukaryotic genomes involves cytosine methylation at the C5 position, typically occurring at cytosine-guanine dinucleotides (CpG) (Suzuki and Bird 2008). CpGs are sparsely distributed in most regions of the genome and densely distributed in only a few regions of the genome, and among these densely distributed regions of CpGs, the regions that satisfy certain criteria for the density and proportion of CpGs are known as CpG islands (CGIs) (Bird 2002; Illingworth and Bird 2009). Genes associated with CpG islands in their promoter regions are more likely to have their expression regulated by methylation levels. An analysis of CpG distribution across the human genome shows that 72% of gene promoter regions are CpG-rich. These genes exhibit expression in most tissues (Saxonov et al. 2006), thereby underscoring the pivotal role of DNA methylation in governing genome expression levels and highlighting that external factors, such as pathogen infections, which can induce abnormal DNA methylation in the human genome, often exert global effects.

### DNMTs in humans

DNMTs typically consist of two distinct structural domains: a regulatory structural domain at the N-terminus and a catalytic structural domain at the C-terminus. The C-terminal catalytic structural domain is highly evolutionarily conserved, primarily because the evolutionarily significant

motifs of DNMT are predominantly situated in this region (Wilson 1992). These distinctive motifs of DNMT encompass motif I, motif IV, motif VI, motif IX, and motif X. Among these motifs, motif IV plays a central role in catalytic activity, mediating the nucleophilic attack on cytosine C6 to initiate the methylation reaction (Du et al. 2016). Six classes of DNMTs have been identified in eukaryotes based on these conserved motifs: DNMT1, DNMT2, DNMT3, DNMT4, DNMT5, and DNMT6, with the first three being widely distributed in animals, and the latter three identified in algae and fungi (Huff and Zilberman 2014). The human genes encode five DNMTs: DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L. While all these DNMTs can introduce methyl groups from SAM into nucleic acids through a base-flipping mechanism, they can be categorized into at least two groups based on their temporal activity: “de novo” DNA methylases and “maintenance” DNA methylases (Riggs 1975). DNMT3A and DNMT3B, as “de novo” DNA methylases, are responsible for adding methylation marks to previously unmodified DNA (Fig. 2a). The “de novo” catalytic function of DNMT3A and DNMT3B is particularly pronounced during embryonic and extraembryonic development (Smith et al. 2012). DNMT3L, a truncated form of DNMT3, lacks catalytic activity due to the absence of a portion of the N-terminal regulatory structural domain and certain motifs in the C-terminal catalytic structural domain. Despite its lack of catalytic activity, DNMT3L is a vital non-enzymatic cofactor for DNMT3A and DNMT3B during “de novo” methylation. Two DNMT3L molecules form a complex with two DNMT3A molecules, enhancing their affinity for DNA and thereby increasing the efficiency of DNMT3A in catalyzing DNA methylation. This complex is crucial in establishing genetic imprinting in gametes (Bourc’his et al. 2001; Jia et al. 2007). DNMT3L is also



**Fig. 2** “De novo” versus “maintenance” DNA methylation. **(a)** Catalyzed by DNMT3a/3b, the methyl group of SAM is transferred to the cytosine of unmethylated CpG dinucleotides in a process known as “de novo” DNA methylation. **(b)** When double-stranded DNA containing methylated CpG dinucleotides is used as a template strand for DNA synthesis, the CpG dinucleotides in the newly synthesized

daughter DNA strand (green) at positions corresponding to the template strand are catalyzed by DNMT1 to add methyl, resulting in the double-stranded daughter DNA replicating the DNA methylation pattern of the template strand accurately, which is known as the process of “maintaining” DNA methylation

essential for DNMT3B to establish a distinct DNA methylation pattern in placental progenitor cells (Smith et al. 2017). It is worth noting that certain catalytically inactive isoforms of DNMT3B, generated through selective splicing, can also act as cofactors similar to DNMT3L under specific circumstances to promote DNA methylation (Duymich et al. 2016).

Compared to “de novo” DNA methylation, the process of “maintenance” DNA methylation appears to be more complex (Fig. 2b). DNA methylation is an important genetic marker that regulates various genomic processes, including transcription and splicing. Consequently, replicating methylation patterns from parental DNA to offspring DNA is essential to maintain genomic stability and cell specificity. However, the raw material required for DNA replication, dNTP, is typically unmethylated. If the parental methylation mark is not copied into the zygote DNA before the initiation of the next S phase following replication, the methylation mark can be lost, leading to dysregulation of gene expression. Therefore, to preserve genome stability, cells require a DNA methyltransferase capable of introducing methyl groups into the newly replicated daughter DNA strands – the “maintenance” DNA methyltransferase. The concept of “maintenance” DNA methylases was hypothesized in the

1970s (Holliday and Pugh 1975). In the 1980s, Reuven Stein and Arthur Weissbach demonstrated that DNMT1 exhibits preferential catalytic activity for hemimethylated sites (Stein et al. 1982; Pedrali-Noy and Weissbach 1986). Although DNMT1 can also mediate methylation of unmethylated CpG dinucleotides in vitro, its activity in this regard is only a fraction (1/50–1/5) of what it achieves when catalyzing hemimethylated substrates (Yoder et al. 1997; Pradhan et al. 1999). Further supporting evidence for the “maintenance” role of DNMT1 was reported by Chen et al. 2003 who found that overexpression of DNMT1 in the absence of DNMT3A and DNMT3B did not induce de novo methylation in mouse embryonic stem cells (Chen et al. 2003). DNMT2 possesses the characteristic catalytic structural domain found in DNMT family members. However, it lacks the N-terminal regulatory structural domain in other family members, and its specific function needs to be more clearly defined.

Although it is expected to categorize DNMTs in the human body as “de novo” and “maintenance” methyltransferases based on distinct functional roles, this classification appears to oversimplify the complex and multifaceted roles DNMTs play in an organism. Notably, the well-established “de novo” methyltransferase, DNMT3, has also been found

to maintain methylation patterns in differentiated cells (Arand et al. 2012; Liao et al. 2015). Hence, the current classification may need to capture the full range of DNMT functions within the organism accurately. The precise *in vivo* roles of DNMTs warrant further investigation and further research into the molecular mechanisms of DNA methylation, and assessing the subtle changes in genome methylation patterns that occur during various stages of growth and development could provide valuable insights, which may, in turn, offer essential clues for the development of more sophisticated, stable and effective therapeutic strategies targeting DNA methylation in various diseases.

### DNA methylation during individual development

DNA methylation is highly dynamic during mammalian embryogenesis, and the genome's methylation pattern undergoes reprogramming, particularly during the germ cell and pre-implantation stages of development. However, the genome's methylation pattern remains unaltered and heritable after somatic differentiation. In somatic genomes, the promoters of housekeeping genes typically feature unmethylated CpG sites, while CpG sites in the non-regulatory regions are mostly methylated. This methylation pattern is usually established early and stably inherited within cells of the same tissue (Bird 2002; Suzuki and Bird 2008). In addition to this relatively stable DNA methylation pattern, minor alterations in CpG methylation status play a role in certain intricate regulatory events within the cell (Weber et al. 2005). Modern studies have challenged the traditional Mendelian theory of inheritance, which posits that each parent transmits an equal set of chromosomes to the offspring, resulting in equal genetic expression from both parents. Phenomena such as X-chromosome inactivation (XCI) and chromosome imprinting in mammals demonstrate that offspring DNA does not carry equal genetic information from both parents. Both X-chromosome inactivation and chromosome imprinting involve differential DNA methylation, especially in the case of X-chromosome inactivation (Lee 2003). Methylation tags are completely removed from the genome at the time of primary germ cell formation to reduce the probability of mutations in future embryonic cells. Subsequent formation of new methylation patterns from scratch by DNMT3A and DNMT3L leads to gender-specific gamete methylation patterns (Gopinathan and Diekwisch 2022). At the end of gametogenesis, approximately 80% of genomic CpGs in sperm are methylated, with a genomic distribution similar to that of somatic cells (Wang et al. 2014). However, after fertilization, the loss of methylation imprinting in the paternal genome was completed during the first six hours of fertilised egg formation (Li et al. 2018), reaching a minimum at the blastocyst stage (Reik et al. 2001).

Methylation reprogramming of genomic DNA during this period may be relevant to establishing cellular totipotency. Re-establishment of methylation patterns occurs at the blastocyst stage and is mediated by DNMT3A and DNMT3B (Breton-Larrivee et al. 2019). During the blastula stage, the inner cell mass forms, giving rise to the embryo. Subsequently, as the embryo implants in the endometrium, DNMT3B-mediated DNA methylation becomes active. This process coincides with the initiation of somatic cell differentiation and leads to the silencing of CGI promoters associated with germline-specific genes (Borgel et al. 2010; Auclair et al. 2014). DNA methylation during this developmental phase is closely associated with the loss of cellular plasticity and lineage restriction. Since the introduction of the first DNMT1 knockout mouse in 1992, our understanding of the roles of DNMTs in individual growth and development has advanced significantly. For example, deleting DNMT1 leads to embryonic death (Li et al. 1992). Subsequent research has elucidated the functions of DNMT2 and DNMT3. Deletion of DNMT3A leads to impaired postnatal development in mice, while DNMT3B deletion results in direct embryonic death (Okano et al. 1999). The findings of Andrews' team reveal the specific mechanism by which DNMT3B knockdown leads to embryonic death—loss of DNMT3B results in de-repression of germline genes in trophoblast lineages and impaired formation of the maternal-foetal interface in the placental labyrinth (Andrews et al. 2023). Although DNMT2 deletion is not lethal, it does cause abnormal hematopoiesis in mice (Tuorto et al. 2015). A recent study has shown that 5-hydroxymethylcytosine (5hmC) may play an important role in regulating gene expression in the placenta and that 5hmC also plays a dynamic role in the differentiation of trophoblast cells during pregnancy (Vasconcelos et al. 2023). These findings underscore the essential role of DNA methylation in biological development. Apart from its role in establishing cellular totipotency during embryonic development and governing the generation of cellular lineages and restriction genes, DNA methylation is a critical mechanism for repressing transposons. Transposons, unique DNA sequences capable of autonomous replication and displacement, constitute about 44% of the human genome. Typically, transposon insertions have harmful effects on the host. DNA methylation temporarily inhibits transposable element activity and facilitates permanent genetic inactivation through mutagenic deamination. Additionally, DNA methylation helps maintain genomic stability by limiting recombination between non-allelic copies of transposable elements that share high sequence similarity (Greenberg and Bourc'his 2019). Studies conducted by Mireia Jordà's team, which revealed a positive correlation between increased levels of chromosomal rearrangements and genome-wide



hypomethylation in various human cancers (Jorda et al. 2017), provide further support for the idea that DNA methylation constrains gene rearrangements, implying a potential role for DNA methylation in the development of diseases.

### DNA methylation in human diseases

The reprogramming of genomic methylation patterns is essential during specific periods such as embryonic development and is necessary for proper development. After somatic cell differentiation, DNA methylation patterns are adjusted to finely regulate specific events within the cell, typically involving only a small set of genes. However, large-scale changes in genome methylation patterns, unrelated to developmental needs, are often linked to human diseases. Different diseases can have distinct effects on host genome methylation patterns, resulting in significant variations in the observed disease manifestations. In the 1980s, researchers noticed that cancer cells exhibited abnormal genome hypomethylation. Conversely, hypermethylation of DNA at the promoters of tumor suppressor genes (TSGs) is common, leading to improper gene expression (Fardi et al. 2018). Hypermethylation of TSG affects various intracellular biological processes: cell cycle, apoptosis, DNA repair, etc. (Pan et al. 2021). This phenomenon has led researchers to speculate that altered methylation patterns may drive tumor formation. Additionally, tumor cells often exhibit the silencing of cell adhesion regulators such as CDH1 (E-cadherin) (Grady et al. 2000) and CDH13 (H-cadherin) (Kim et al. 2005), suggesting that aberrant DNA methylation may not only play a role in tumor initiation but also contribute to the acquisition of characteristics like migration and invasion in tumor cells. DNMT has a dual role in tumorigenesis, acting as both an oncogene and tumor suppressor gene. For example, DNMT3B is an oncogene in breast, liver, colon, and ovarian cancers but acts as an oncogene in lymphomas; DNMT3a is an oncogene in pituitary adenomas, lung cancer and melanoma, but acts as a TSG in lung cancer and chronic lymphocytic leukaemia (Zhang et al. 2020). Characteristic alterations in DNA methylation levels have been found in peripheral immune cells from a variety of cancers, such as hepatocellular carcinoma (Zhang et al. 2018), breast cancer (Wang et al. 2023), etc. As the elderly population continues to grow, neurodegenerative diseases such as Alzheimer's disease (AD), Huntington's chorea (HD), Parkinson's disease (PD), and amyotrophic lateral sclerosis are gaining widespread attention due to their increasing prevalence, considering that the most significant risk factor for these diseases is age. As individuals age, their genomes accumulate epigenetic changes that disrupt gene expression, leading to tissue dysfunction, reduced regenerative capacity, and an increased risk of developing neurodegenerative diseases (Lu

et al. 2020). Changes in DNA methylation patterns during aging underlie individual senescence. Methylation levels of ribosomal DNA in somatic cells increase with age, leading to impaired ribosome biogenesis, which in turn triggers kernel stress and, ultimately, aging-related diseases (Wang et al. 2015). In addition, methylation of rDNA transcription units, including the upstream control element (UCE), core promoter, 18S rDNA, and 28S rDNA in spermatozoa, increased significantly with donor age (Potabattula et al. 2020). In addition to rDNA, the level of CpG island methylation in the promoter regions of genes associated with regulating cell growth and development increases with age (Pirazzini et al. 2021). Apart from cancer and neurodegenerative diseases, abnormal alterations in genomic DNA methylation patterns are observed in various human diseases, including those related to the cardiovascular system, autoimmune responses, inflammation, and metabolism. These alterations generally involve switching genes from their normal expression state to being turned off or vice versa. However, the specific genes and epigenetic information affected can vary among diseases. The strong link between DNA methylation and disease has driven research into targeted drugs and therapies. However, due to the lack of research to identify effective candidate genes for DNA methylation modification, epigenetic therapies currently only have some application in cancer. Additionally, epigenetic changes are closely tied to immune and metabolic processes in the body. Non-targeted approaches often result in genome-wide alterations in epigenetic patterns, which can lead to serious side effects. Therefore, future research should focus on identifying specific epigenetic factors and their underlying molecular mechanisms, which could provide a more comprehensive understanding of DNA methylation's role in regulating various diseases, enabling the development of more targeted and effective therapies.

### DNA methylation and *Mycobacterium tuberculosis*

Epigenetic regulation in eukaryotes can operate at various levels, including nucleosome localization, DNA methylation, histone variation, and histone modification. In contrast, bacteria lack components such as nucleosomes and histones, relying primarily on DNA methylation for epigenetic regulation. DNA methylation has been observed in over 93% of prokaryotic genomes (Blow et al. 2016). Unlike eukaryotes, prokaryotic genomes primarily employ 6 mA as their main mode of DNA methylation. *Mycobacterium tuberculosis*, from approximately 70,000 ago in Africa (Comas et al. 2013), has evolved extensively from a common environmental microorganism into one of the most successful pathogens. DNA methylation, a form of epigenetic regulation present

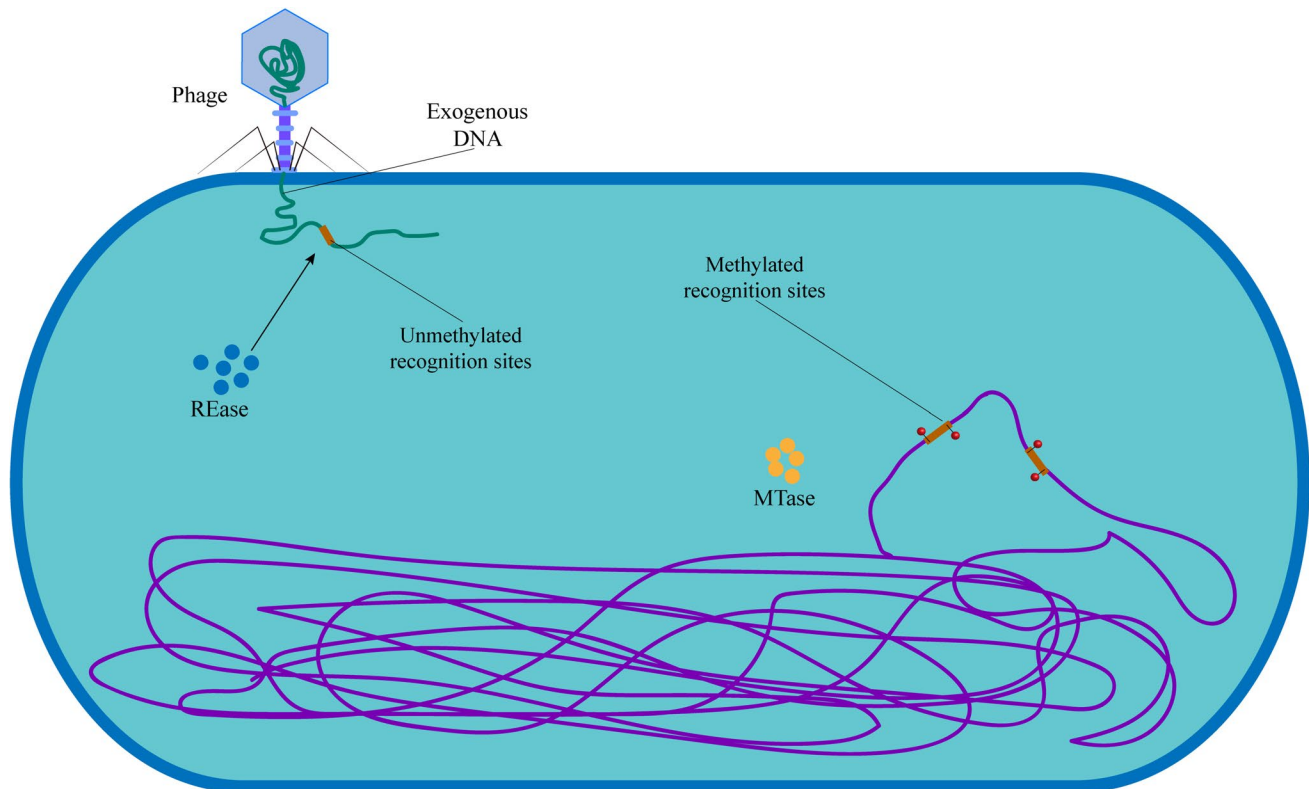
in both prokaryotes and eukaryotes, has played a significant role in both the evolution of *Mycobacterium tuberculosis* itself and in the modulation of the immune response during *Mycobacterium tuberculosis* infection in the host.

### DNA methylation in Mtb

The combination of mycobacterial latency and drug resistance represents the two major contributors to the devastating impact of tuberculosis, presenting a significant challenge to the academic community. The acquisition of latent properties by Mtb can be traced back to a time before it had evolved into a pathogen. It is suggested that a critical event in the extensive evolution of *Mycobacterium tuberculosis* was its development of the ability to persist within certain protists that prey on bacteria (Jang et al. 2008). While genetic mutations may have contributed to Mtb's tolerance to these internal conditions, the role of DNA methylation regulation in the evolution of *Mycobacterium tuberculosis* appears to be more crucial. DNA methylation offers a faster and more adaptable mechanism for altering gene expression in Mtb, enabling it to acquire specific resistances upon ingestion. DNA methylation is a primary mode of epigenetic regulation in bacteria. This capability to persist within the host also raises the possibility of subsequent infections by *Mycobacterium tuberculosis* in mammals, where the bacterium can persist and multiply. The academic community remains deeply concerned about Mtb's ability to persist within the host organism, a trait commonly called "latency." Through extensive research, we have come to understand that tuberculosis often undergoes a latent phase within the host that can span several decades. During this period, Mtb exhibits characteristics such as slow growth, enhanced resistance to oxidative stress, and reduced sensitivity to drugs (Kinsella et al. 2021). However, when the immune system becomes compromised due to factors like old age or other illnesses (i.e., HIV infection or type II diabetes), the previously suppressed *Mycobacterium tuberculosis* becomes reactivated, contributing to the local epidemiological trends of tuberculosis in a given region (Cadena et al. 2017). The acquisition of latency represents a foundational adaptation for Mtb's survival. In the harsh natural environment, successful species evolution requires changes in gene expression to develop better traits for adaptation and, perhaps more crucially, the preservation of genetic material stability. This stability ensures the consistent inheritance of advantageous traits acquired by parents from the external environment, passing them down to offspring and securing the species' continuity. In addition to assisting Mtb in altering genomic expression patterns in response to external stresses, DNA methylation modifications also play a pivotal role in maintaining the stability of Mtb's genetic material. Prokaryotic DNA methyltransferases often collaborate with restriction

nucleic acid endonucleases, forming a system known as the restriction-modification (RM) system (Anton and Roberts 2021). This system is considered a primitive immune defense mechanism. DNA methyltransferases safeguard their chromosomes and certain beneficial exogenous DNAs from degradation by preventing restriction endonucleases from binding to DNA by adding a methylation imprint at the binding site of the restriction endonuclease (Fig. 3). This primitive defense maintains the stability of the Mtb genome and enhances Mtb survival to some extent. DNA methyltransferases are sometimes referred to as DNA protectors. In contrast, restriction endonucleases degrade exogenous DNA lacking specific methylation marks. This division of labor maintains the *Mycobacterium tuberculosis* genome's stability while providing some protection against phage invasion. It is worth noting that not all exogenous DNA is harmful to bacteria. Horizontal gene transfer (HGT) has played a significant role in prokaryotic evolution. The ancestors of *Mycobacterium tuberculosis* acquired genes, including those encoding transferase enzymes and genes adapted to anaerobic conditions, from other bacteria (Boritsch et al. 2014; Reva et al. 2015), which might have contributed to the ambiguity of Mtb's evolution into a specialized pathogen capable of persisting in the host's cytosol. Plasmids often serve as carriers for these mobile elements, containing genes essential for bacterial survival and "helper" genes that degrade toxic substances or provide the bacteria with new metabolic capabilities (Attere et al. 2017; Ares-Arroyo et al. 2018). The array of advantageous traits encoded by plasmids expands the range of environmental conditions where the host can thrive and plays a crucial role in bacterial clade evolution (Rodriguez-Beltran et al. 2021). However, it is important to note that while HGT has been instrumental in Mtb's evolution, transferring exogenous genes beyond a certain range can be detrimental to one's genome. Therefore, RM systems often selectively add methylation markers to exogenous DNA entering the cytosol, which, to some extent, restricts the HGT of mobile elements, maintaining the relative stability of the genome (Murray 2002).

The study of DNA methylation in immunity to *Mycobacterium tuberculosis* infection began with the discovery of methylation imprinting in the Mtb genome in 1925. Three DNA methyltransferases responsible for 6 mA modifications—MamA, MamB, and Hsdm—have since been identified in the Mtb genome (Zhu et al. 2016; Phelan et al. 2018). In addition to regulating Mtb's expected growth, these enzymes mediate the methylation of relevant genes, which plays a crucial role in Mtb's intracellular survival and drug resistance. A study by Scarlet S. Shell revealed the presence of transcription start sites (TSS) for genes like Rv0102, Rv0142, corA, and whiB7 at 4 or 5 base pairs from the MamA methylation site. Deletion of MamA results in reduced viability of Mtb under hypoxic conditions (Shell



**Fig. 3** Defence mechanisms of the restriction-modification (R-M) system. The recognition site of Mtb's own genome is methylated by DNA methyltransferase (MTase), thus avoiding degradation by

restriction endonucleases (REase). Exogenous DNA (e.g. phage DNA) is degraded by REase cleavage because the recognition site is not methylated

et al. 2013). Given that *Mycobacterium tuberculosis* is an aerobic bacterium specialized for survival, it must possess the capability to adapt to hypoxic conditions, which is especially important for Mtb's latency within an intracellular environment where oxygen levels are lower than in the external environment. MamA-mediated DNA methylation of relevant genes is crucial for Mtb to thrive in hypoxic conditions (Shell et al. 2013). In addition to Mtb's ability to cope with hypoxia, methyltransferases have been linked to *Mycobacterium tuberculosis*' resistance to antibiotics. Hongqian Chu's research team observed a significant reduction in the growth rate of the *Mycobacterium tuberculosis* multidrug-resistant strain 11,826 when its Hsdm gene was knocked out, varying degrees of increased sensitivity to drugs such as rifampicin, levofloxacin, and ethambutol were also observed (Chu et al. 2021). Bioinformatics analysis of the Mtb genome revealed that many Hsdm-modified genes were enriched in redox and energy metabolism-related pathways (Hu 2021). Previous reports have demonstrated that bacterial susceptibility to antibiotics is closely tied to the redox state (Lobritz et al. 2015). This suggests that the deletion of Hsdm results in an abnormal methylation status of Mtb metabolism-related genes, impairing the bacterium's normal metabolic function within host cells. Most antibiotics work by inhibiting

or killing bacteria by suppressing their energy production. Therefore, the deletion of Hsdm enhances the inhibitory effect of antibiotics, potentially making the bacteria more susceptible to these drugs.

The methylation-modified locus of 6 mA methyltransferase from *Mycobacterium tuberculosis* contains genes closely related to Mtb's energy metabolism (such as NuoI, ctaE, fadD, etc.); knockdown of this enzyme has been demonstrated to inhibit Mtb's ability to survive in low-oxygen environments and increase its sensitivity to drugs (Chu et al. 2021). These findings suggest that DNA methylation holds significant research potential for investigating drug resistance and latent properties of Mtb. It may be a critical factor in the future eradication of TB. However, it is unfortunate that current research on DNA methylation in TB still needs to be improved. Many aspects of DNA methylation during Mtb infection remain unknown and unexplored, and there is a considerable distance to cover before DNA methylation can be applied in the clinical diagnosis and treatment of TB.

### DNA methylation in the host during Mtb infection

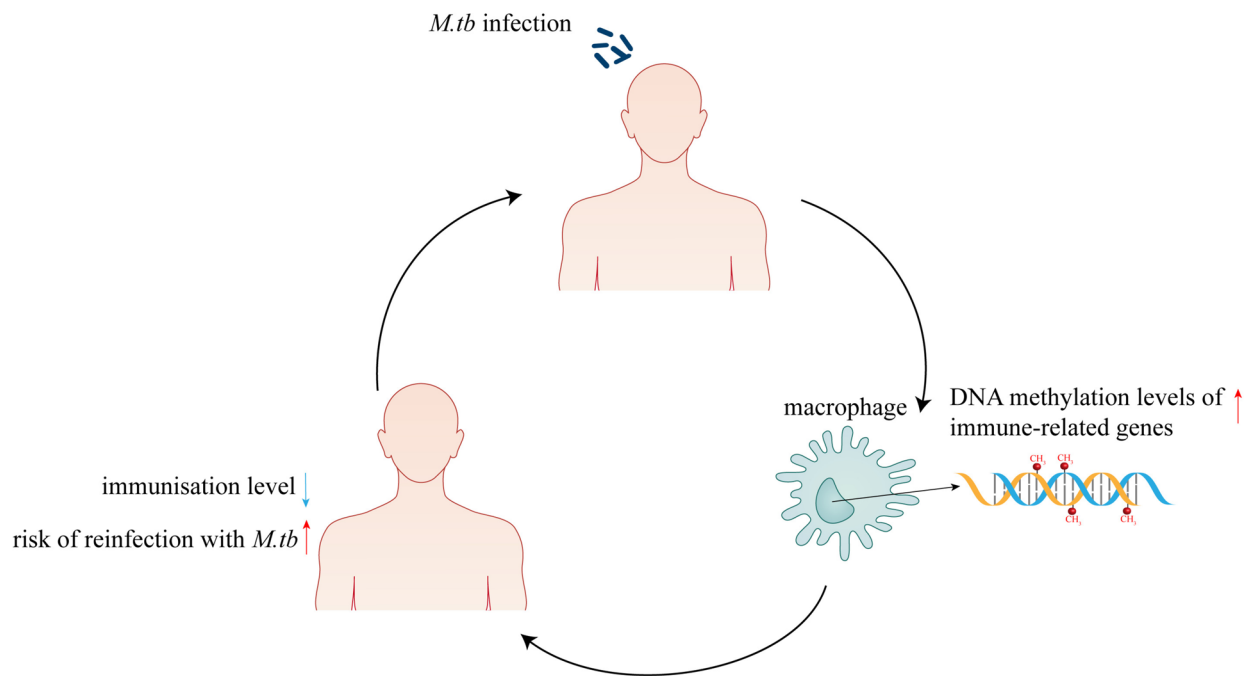
Studies investigating the interaction between pathogenic bacteria and mammals have revealed that pathogenic



bacteria trigger immune responses and lead to changes in gene expression and specific cellular signaling pathways (Bhavsar et al. 2007). DNA methylation, a regulatory mechanism that plays a crucial role in various biological processes of the immune system, is also involved in the infection processes of many pathogenic bacteria, leading to alterations in the host genome's methylation status. For example, *Porphyromonas gingivalis* infection affects gingival cytokine release through DNA methylation (Drury and Chung 2015), and *Helicobacter pylori* infection leads to abnormal DNA methylation in the gastric mucosa (Maekita et al. 2006). The impact of Mtb infection on the host genome's methylation status has been a significant area of research due to the long-standing battle between humans and Mtb. As the first line of immune defense against Mtb infection, macrophages activate various signaling pathways to initiate immune responses against invading pathogens. Toll-like receptors (TLRs) expressed on macrophages recognize pathogen-associated molecular patterns (PAMPs) on Mtb and subsequently induce the production of immune-related cytokines such as tumor necrosis factor (TNF) and type I interferon (IFN) (Xu et al. 2014). During *Mycobacterium tuberculosis* infection, cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 beta (IL-1 $\beta$ ) play pivotal roles in the host immune response. When Mtb is inhaled into the body, it binds to phagocytic receptors and gains entry into alveolar macrophages, which are subsequently recruited into the bloodstream (Bhatt and Salgame 2007). Following the phagocytosis of Mtb, these alveolar macrophages migrate to the interstitial spaces within the lungs. This migration is accompanied by the infiltration of other immune cells, leading to the formation of structures known as granulomas (Cohen et al. 2018). Lung granulomas are commonly regarded as the focal point of the immune response against Mtb. However, animal model studies have revealed that individual granulomas exhibit varying abilities to control Mtb based on their unique microenvironments. While some granulomas can completely clear Mtb, the overall outcome typically results in a state of equilibrium where the immune system manages Mtb sustainably while allowing it to persist for an extended period (Cohen et al. 2022). Individuals in this state of equilibrium are referred to as latently infected with Mtb. Although there are no obvious clinical signs, once active TB develops in a sudden situation, these individuals are highly likely to transmit TB unknowingly. Genes impacted by DNA methylation changes induced by Mtb are primarily related to the immune system. TB patients typically exhibit a diminished immune response compared to the general population. For instance, one study found that Mtb infection led to methylation of the IL-6 promoter, potentially resulting in reduced IL-6 levels (Zheng et al. 2016). IL-6 is associated with the differentiation of macrophages and cytotoxic T-cells, and a decrease in this cytokine can facilitate

Mtb survival within host cells. Another study compared methylation levels at 20 CpG sites in the promoter region of the Toll-like receptor 2 (TLR2) gene in TB patients and the normal population. TB patients exhibited higher methylation levels at five sites, leading to reduced TLR2 protein expression in monocytes (Chen et al. 2014). TLR2 plays a crucial role in innate immunity against Mtb infection. Bobak et al. observed aberrant DNA methylation in guinea pigs' spleen and lung genomes four days after Mtb infection. Bioinformatics analyses showed that hypermethylated genes were enriched in immune-related signaling pathways such as the PI3K-AKT signaling pathway, the MAPK signaling pathway, and the mTOR signaling pathway (Bobak et al. 2022). Additionally, the risk of reinfection with Mtb in the TB-healed group was significantly higher than in the healthy population (about 13 times higher than in normal individuals) (Millet et al. 2013). Furthermore, DiNardo et al. discovered that TB patients exhibit a hyporesponsive immune phenotype, characterized by reduced immune proliferation and diminished production of cytokines in response to Mycobacterium and mitogens (DiNardo et al. 2020). Genomic DNA methylation sequencing revealed these phenomena were linked to DNA hypermethylation of key immune genes and pathways. This hypermethylation persisted even after the successful completion of treatment. These findings indicate that Mtb can suppress the host immune response through DNA methylation regulation. The detrimental methylation changes imposed by Mtb persist not only during infection but also post-treatment, which can partly explain why bacterial culture tests for most TB patients typically become negative after two months of antibiotic treatment, with treatment typically lasting up to six months.

*Mycobacterium tuberculosis* has developed various strategies to enhance its survival within the host. Upon infecting and successfully parasitizing the host, Mtb faces pressure from the host's immune system, prompting it to evolve tactics like limiting the production of antimicrobial peptides, escaping to the cytosol, and inhibiting cellular autophagy to evade host immune clearance (Liu et al. 2017). These interference mechanisms employed by Mtb in host cells are often associated with the abnormal DNA methylation patterns it induces in the host genome. The DNA methylation imprints triggered by Mtb infection facilitate intracellular survival and dampen the host's immune response, prolonging the risk of reinfection long after TB healing (Fig. 4). This deleterious cycle disrupts the host's immune system, increasing susceptibility to Mtb and a variety of other pathogens and resulting in much higher individual mortality. Rapid and precise removal of Mtb-induced abnormal DNA methylation in the host genome is crucial to break this cycle. It holds great promise for developing more effective therapeutic and diagnostic methods.



**Fig. 4** The vicious cycle of *Mycobacterium tuberculosis* infection

## Summary

The lag in TB diagnostics significantly hampers progress in the TB elimination strategy. TB treatment approaches have remained relatively unchanged for decades, with the mainstream methods currently in use, such as BCG and antibiotic regimens, dating back at least 40 years (Gagneux 2018). While these traditional TB treatments have been effective against common strains of *Mycobacterium tuberculosis*, they are less effective in combating the drug-resistant mutant strains that pose a significant global threat. There is a growing concern that drug-resistant Mtb strains potentially lead to a new global TB crisis. WHO-recommended treatment regimens for drug-resistant TB remain less effective for XDR-TB. Understanding the mechanisms behind *Mycobacterium tuberculosis*'s rapid adaptation to antibiotic treatment and the host's immune responses is crucial. This knowledge is essential for advancing strategies to combat tuberculosis. In recent years, with the continuous development of high-throughput sequencing technology, the role of DNA methylation in Mtb's interactions with the host has become a focal point of TB research. Evidence suggests that DNA methylation is intricately linked to Mtb's metabolism and virulence. However it is clearly impractical to screen for latent TB infection worldwide by means of DNA sequencing. This approach consumes substantial resources, especially in TB's high-burden countries, and requires significant time. Moreover, it does not contribute significantly

to diagnosing dynamic Mtb infections. Nonetheless, the ongoing advancements in high-throughput sequencing technology can be harnessed to facilitate the study of DNA methylation during Mtb infection. This technology offers an opportunity to gain deeper insights into the specific mechanisms of methylation modification in both Mtb itself and the host genome. Such insights hold immense significance in enhancing the drug sensitivity of bacterial strains, identifying more promising drug targets, and developing cost-effective and user-friendly diagnostic methods with high sensitivity and specificity, which may even contribute to solving the challenge of Mtb latency in the future.

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**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Conflict of interests** The authors have no relevant financial or non-financial interests to disclose.

## References

- Andrews S et al (2023) Mechanisms and function of de novo DNA methylation in placental development reveals an essential role for DNMT3B. *Nat Commun* 14:371. <https://doi.org/10.1038/s41467-023-36019-9>
- Anton BP, Roberts RJ (2021) Beyond restriction modification: epigenomic roles of DNA methylation in prokaryotes. *Annu Rev Microbiol* 75:129–149. <https://doi.org/10.1146/annurev-micro-040521-035040>
- Arand J et al (2012) In vivo control of CpG and non-CpG DNA methylation by DNA methyltransferases. *PLoS Genet* 8:e1002750. <https://doi.org/10.1371/journal.pgen.1002750>
- Ares-Arroyo M et al (2018) PCR-based analysis of *cole1* plasmids in clinical isolates and metagenomic samples reveals their importance as gene capture platforms. *Front Microbiol* 9:469. <https://doi.org/10.3389/fmicb.2018.00469>
- Attere SA, Vincent AT, Paccaud M, Frenette M, Charette SJ (2017) The role for the small cryptic plasmids as moldable vectors for genetic innovation in *Aeromonas salmonicida* subsp. *salmonicida*. *Front Genet* 8:211. <https://doi.org/10.3389/fgene.2017.00211>
- Auclair G, Guibert S, Bender A, Weber M (2014) Ontogeny of CpG island methylation and specificity of DNMT3 methyltransferases during embryonic development in the mouse. *Genome Biol* 15:545. <https://doi.org/10.1186/s13059-014-0545-5>
- Bhatt K, Salgame P (2007) Host innate immune response to *Mycobacterium tuberculosis*. *J Clin Immunol* 27:347–362. <https://doi.org/10.1007/s10875-007-9084-0>
- Bhavsar AP, Guttman JA, Finlay BB (2007) Manipulation of host-cell pathways by bacterial pathogens. *Nature* 449:827–834. <https://doi.org/10.1038/nature06247>
- Bird A (2002) DNA methylation patterns and epigenetic memory. *Genes Dev* 16:6–21. <https://doi.org/10.1101/gad.947102>
- Blow MJ et al (2016) The epigenomic landscape of prokaryotes. *PLoS Genet* 12:e1005854. <https://doi.org/10.1371/journal.pgen.1005854>
- Bobak CA et al (2022) Increased DNA methylation, cellular senescence and premature epigenetic aging in guinea pigs and humans with tuberculosis. *Aging (albany NY)* 14:2174–2193. <https://doi.org/10.18632/aging.203936>
- Borgel J et al (2010) Targets and dynamics of promoter DNA methylation during early mouse development. *Nat Genet* 42:1093–1100. <https://doi.org/10.1038/ng.708>
- Boritsch EC, Supply P, Honore N, Seemann T, Stinear TP, Brosch R (2014) A glimpse into the past and predictions for the future: the molecular evolution of the tuberculosis agent. *Mol Microbiol* 93:835–852. <https://doi.org/10.1111/mmi.12720>
- Bourc'his D, Xu GL, Lin CS, Bollman B, Bestor TH (2001) Dnmt3L and the establishment of maternal genomic imprints. *Science* 294:2536–2539. <https://doi.org/10.1126/science.1065848>
- Breton-Larrievée M, Elder E, McGraw S (2019) DNA methylation, environmental exposures and early embryo development. *Anim Reprod* 16:465–474. <https://doi.org/10.21451/1984-3143-AR2019-0062>
- Cadena AM, Fortune SM, Flynn JL (2017) Heterogeneity in tuberculosis. *Nat Rev Immunol* 17:691–702. <https://doi.org/10.1038/nri.2017.69>
- Chen T, Ueda Y, Dodge JE, Wang Z, Li E (2003) Establishment and maintenance of genomic methylation patterns in mouse embryonic stem cells by Dnmt3a and Dnmt3b. *Mol Cell Biol* 23:5594–5605. <https://doi.org/10.1128/MCB.23.16.5594-5605.2003>
- Chen YC et al (2014) Aberrant Toll-like receptor 2 promoter methylation in blood cells from patients with pulmonary tuberculosis. *J Infect* 69:546–557. <https://doi.org/10.1016/j.jinf.2014.08.014>
- Chu H, Hu Y, Zhang B, Sun Z, Zhu B (2021) DNA methyltransferase HsdM induce drug resistance on *Mycobacterium tuberculosis* via multiple effects. *Antibiotics (basel)*. <https://doi.org/10.3390/antibiotics10121544>
- Cohen SB et al (2018) Alveolar macrophages provide an early mycobacterium tuberculosis niche and initiate dissemination. *Cell Host Microbe* 24(439–446):e434. <https://doi.org/10.1016/j.chom.2018.08.001>
- Cohen SB, Gern BH, Urdahl KB (2022) The tuberculous granuloma and preexisting immunity. *Annu Rev Immunol* 40:589–614. <https://doi.org/10.1146/annurev-immunol-093019-125148>
- Comas I et al (2013) Out-of-Africa migration and Neolithic coexpansion of *Mycobacterium tuberculosis* with modern humans. *Nat Genet* 45:1176–1182. <https://doi.org/10.1038/ng.2744>
- Cruijssen M, Lubbert M, Wijermans P, Huls G (2014) Clinical results of hypomethylating agents in aml treatment. *J Clin Med* 4:1–17. <https://doi.org/10.3390/jcm4010001>
- DiNardo AR et al (2020) DNA hypermethylation during tuberculosis dampens host immune responsiveness. *J Clin Investig* 130:3113–3123. <https://doi.org/10.1172/JCI134622>
- Drury JL, Chung WO (2015) DNA methylation differentially regulates cytokine secretion in gingival epithelia in response to bacterial challenges. *Pathog Dis* 73:1–6. <https://doi.org/10.1093/femspd/ftu005>
- Du Q, Wang Z, Schramm VL (2016) Human DNMT1 transition state structure. *Proc Natl Acad Sci U S A* 113:2916–2921. <https://doi.org/10.1073/pnas.1522491113>
- Du Y et al (2022) Relationship between DNA methylation profiles and active tuberculosis development from latent infection: a pilot study in nested case-control design. *Microbiol Spectr* 10:e0058622. <https://doi.org/10.1128/spectrum.00586-22>
- Duymich CE, Charlet J, Yang X, Jones PA, Liang G (2016) DNMT3B isoforms without catalytic activity stimulate gene body methylation as accessory proteins in somatic cells. *Nat Commun* 7:11453. <https://doi.org/10.1038/ncomms11453>
- Fardi M, Solali S, Farshdousti Hagh M (2018) Epigenetic mechanisms as a new approach in cancer treatment: an updated review. *Genes Dis* 5:304–311. <https://doi.org/10.1016/j.gendis.2018.06.003>
- Gagneux S (2018) Ecology and evolution of *Mycobacterium tuberculosis*. *Nat Rev Microbiol* 16:202–213. <https://doi.org/10.1038/nrmicro.2018.8>
- Gopinathan G, Diekwisch TGH (2022) Epigenetics and early development. *J Dev Biol*. <https://doi.org/10.3390/jdb10020026>
- Grady WM et al (2000) Methylation of the CDH1 promoter as the second genetic hit in hereditary diffuse gastric cancer. *Nat Genet* 26:16–17. <https://doi.org/10.1038/79120>
- Greenberg MVC, Bourc'his D (2019) The diverse roles of DNA methylation in mammalian development and disease. *Nat Rev Mol Cell Biol* 20:590–607. <https://doi.org/10.1038/s41580-019-0159-6>
- Gupta RK et al (2020) Quantitative IFN-gamma release assay and tuberculin skin test results to predict incident tuberculosis. prospective cohort study. *Am J Respir Crit Care Med* 201:984–991. <https://doi.org/10.1164/rccm.201905-0969OC>
- Holliday R, Pugh JE (1975) DNA modification mechanisms and gene activity during development. *Sci* 187:226–232
- Hu X et al (2021) The mycobacterial DNA methyltransferase HsdM decreases intrinsic isoniazid susceptibility. *Antibiotics (basel)*. <https://doi.org/10.3390/antibiotics10111323>
- Huff JT, Zilberman D (2014) Dnmt1-independent CG methylation contributes to nucleosome positioning in diverse eukaryotes. *Cell* 156:1286–1297. <https://doi.org/10.1016/j.cell.2014.01.029>
- Illingworth RS, Bird AP (2009) CpG islands—'a rough guide'. *FEBS Lett* 583:1713–1720. <https://doi.org/10.1016/j.febslet.2009.04.012>
- Jaenisch R, Bird A (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* 33(Suppl):245–254. <https://doi.org/10.1038/ng1089>

- Jang J, Becq J, Gicquel B, Deschavanne P, Neyrolles O (2008) Horizontally acquired genomic islands in the tubercle bacilli. *Trends Microbiol* 16:303–308. <https://doi.org/10.1016/j.tim.2008.04.005>
- Jia D, Jurkowska RZ, Zhang X, Jeltsch A, Cheng X (2007) Structure of Dnmt3a bound to Dnmt3L suggests a model for de novo DNA methylation. *Nature* 449:248–251. <https://doi.org/10.1038/nature06146>
- Jorda M et al (2017) The epigenetic landscape of Alu repeats delineates the structural and functional genomic architecture of colon cancer cells. *Genome Res* 27:118–132. <https://doi.org/10.1101/gr.207522.116>
- Kim JS, Han J, Shim YM, Park J, Kim DH (2005) Aberrant methylation of H-cadherin (CDH13) promoter is associated with tumor progression in primary nonsmall cell lung carcinoma. *Cancer* 104:1825–1833. <https://doi.org/10.1002/cncr.21409>
- Kinsella RL et al (2021) Perspectives and advances in the understanding of tuberculosis. *Annu Rev Pathol* 16:377–408. <https://doi.org/10.1146/annurev-pathol-042120-032916>
- Lee JT (2003) Molecular links between X-inactivation and autosomal imprinting: X-inactivation as a driving force for the evolution of imprinting? *Curr Biol* 13:R242–R254. [https://doi.org/10.1016/s0960-9822\(03\)00162-3](https://doi.org/10.1016/s0960-9822(03)00162-3)
- Li E, Bestor TH, Jaenisch R (1992) Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* 69:915–926. [https://doi.org/10.1016/0092-8674\(92\)90611-f](https://doi.org/10.1016/0092-8674(92)90611-f)
- Li C et al (2018) DNA methylation reprogramming of functional elements during mammalian embryonic development. *Cell Discov* 4:41. <https://doi.org/10.1038/s41421-018-0039-9>
- Liao J et al (2015) Targeted disruption of DNMT1, DNMT3A and DNMT3B in human embryonic stem cells. *Nat Genet* 47:469–478. <https://doi.org/10.1038/ng.3258>
- Liu CH, Liu H, Ge B (2017) Innate immunity in tuberculosis: host defense vs pathogen evasion. *Cell Mol Immunol* 14:963–975. <https://doi.org/10.1038/cmi.2017.88>
- Lobritz MA et al (2015) Antibiotic efficacy is linked to bacterial cellular respiration. *Proc Natl Acad Sci USA* 112:8173–8180. <https://doi.org/10.1073/pnas.1509743112>
- Lu Y et al (2020) Reprogramming to recover youthful epigenetic information and restore vision. *Nature* 588:124–129. <https://doi.org/10.1038/s41586-020-2975-4>
- Maekita T et al (2006) High levels of aberrant DNA methylation in *Helicobacter pylori*-infected gastric mucosae and its possible association with gastric cancer risk. *Clin Cancer Res* 12:989–995. <https://doi.org/10.1158/1078-0432.CCR-05-2096>
- Mazurek GH et al (2005) Guidelines for using the QuantiFERON-TB Gold test for detecting *Mycobacterium tuberculosis* infection, United States. *MMWR Recomm Rep* 54:49–55
- Millet JP et al (2013) Tuberculosis recurrence after completion treatment in a European city: reinfection or relapse? *PLoS ONE* 8:e64898. <https://doi.org/10.1371/journal.pone.0064898>
- Moore LD, Le T, Fan G (2013) DNA methylation and its basic function. *Neuropsychopharmacol* 38:23–38. <https://doi.org/10.1038/npp.2012.112>
- Murray NE (2002) 2001 Fred Griffith review lecture. Immigration control of DNA in bacteria: self versus non-self. *Microbio (reading)* 148:3–20. <https://doi.org/10.1099/00221287-148-1-3>
- Okano M, Bell DW, Haber DA, Li E (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 99:247–257. [https://doi.org/10.1016/s0092-8674\(00\)81656-6](https://doi.org/10.1016/s0092-8674(00)81656-6)
- Pan H et al (2021) Discovery of candidate dna methylation cancer driver genes. *Cancer Discov* 11:2266–2281. <https://doi.org/10.1158/2159-8290.CD-20-1334>
- Pedrali-Noy G, Weissbach A (1986) Mammalian DNA methyltransferases prefer poly(dI-dC) as substrate. *J Biol Chem* 261:7600–7602
- Pero R et al (2011) Chromatin and DNA methylation dynamics of *Helicobacter pylori*-induced COX-2 activation. *Int J Med Microbiol* 301:140–149. <https://doi.org/10.1016/j.ijmm.2010.06.009>
- Phelan J et al (2018) Methylation in *Mycobacterium tuberculosis* is lineage specific with associated mutations present globally. *Sci Rep* 8:160. <https://doi.org/10.1038/s41598-017-18188-y>
- Pirazzini C et al (2021) A geroscience approach for Parkinson's disease: Conceptual framework and design of PROPAG-AGEING project. *Mech Ageing Dev* 194:111426. <https://doi.org/10.1016/j.mad.2020.111426>
- Ponger L, Li WH (2005) Evolutionary diversification of DNA methyltransferases in eukaryotic genomes. *Mol Biol Evol* 22:1119–1128. <https://doi.org/10.1093/molbev/msi098>
- Potabattula R et al (2020) Increasing methylation of sperm rDNA and other repetitive elements in the aging male mammalian germline. *Aging Cell* 19:e13181. <https://doi.org/10.1111/acer.13181>
- Pradhan S, Bacolla A, Wells RD, Roberts RJ (1999) Recombinant human DNA (cytosine-5) methyltransferase. I. expression, purification, and comparison of de novo and maintenance methylation. *J Biol Chem* 274:33002–33010. <https://doi.org/10.1074/jbc.274.46.33002>
- Reik W, Dean W, Walter J (2001) Epigenetic reprogramming in mammalian development. *Science* 293:1089–1093. <https://doi.org/10.1126/science.1063443>
- Reva O, Korotetskiy I, Ilin A (2015) Role of the horizontal gene exchange in evolution of pathogenic *Mycobacteria*. *BMC Evol Biol* 15(Suppl 1):S2. <https://doi.org/10.1186/1471-2148-15-S1-S2>
- Riggs AD (1975) X inactivation, differentiation, and DNA methylation. *Cytogenet Cell Genet* 14:9–25. <https://doi.org/10.1159/000130315>
- Rodriguez-Beltran J, DelaFuente J, Leon-Sampedro R, MacLean RC, San Millan A (2021) Beyond horizontal gene transfer: the role of plasmids in bacterial evolution. *Nat Rev Microbiol* 19:347–359. <https://doi.org/10.1038/s41579-020-00497-1>
- Saxonov S, Berg P, Brutlag DL (2006) A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters. *Proc Natl Acad Sci USA* 103:1412–1417. <https://doi.org/10.1073/pnas.0510310103>
- Shah M, Dorman SE (2021) Latent tuberculosis infection. *N Engl J Med* 385:2271–2280. <https://doi.org/10.1056/NEJMc2108501>
- Shell SS et al (2013) DNA methylation impacts gene expression and ensures hypoxic survival of *Mycobacterium tuberculosis*. *PLoS Pathog* 9:e1003419. <https://doi.org/10.1371/journal.ppat.1003419>
- Smith ZD et al (2012) A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature* 484:339–344. <https://doi.org/10.1038/nature10960>
- Smith ZD et al (2017) Epigenetic restriction of extraembryonic lineages mirrors the somatic transition to cancer. *Nature* 549:543–547. <https://doi.org/10.1038/nature23891>
- Stein R, Gruenbaum Y, Pollack Y, Razin A, Cedar H (1982) Clonal inheritance of the pattern of DNA methylation in mouse cells. *Proc Natl Acad Sci U S A* 79:61–65. <https://doi.org/10.1073/pnas.79.1.61>
- Stein CM et al (2018) Resistance and susceptibility to *Mycobacterium tuberculosis* infection and disease in tuberculosis households in kampala, uganda. *Am J Epidemiol* 187:1477–1489. <https://doi.org/10.1093/aje/kwx380>
- Suzuki MM, Bird A (2008) DNA methylation landscapes: provocative insights from epigenomics. *Nat Rev Genet* 9:465–476. <https://doi.org/10.1038/nrg2341>
- Tuorto F et al (2015) The tRNA methyltransferase Dnmt2 is required for accurate polypeptide synthesis during haematopoiesis. *EMBO J* 34:2350–2362. <https://doi.org/10.15252/embj.201591382>
- Vasconcelos S, Camicais C, de Sousa C, Lopes SM, Marques CJ, Doria S (2023) The role of DNA hydroxymethylation and TET enzymes in placental development and pregnancy outcome. *Clin Epigenet* 15:66. <https://doi.org/10.1186/s13148-023-01483-z>



- Waddington CH (2012) The epigenotype. 1942. *Int J Epidemiol* 41:10–13. <https://doi.org/10.1093/ije/dyr184>
- Wang L et al (2014) Programming and inheritance of parental DNA methylomes in mammals. *Cell* 157:979–991. <https://doi.org/10.1016/j.cell.2014.04.017>
- Wang T et al (2023) A multiplex blood-based assay targeting DNA methylation in PBMCs enables early detection of breast cancer. *Nat Commun* 14:4724. <https://doi.org/10.1038/s41467-023-40389-5>
- Weber M et al (2005) Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. *Nat Genet* 37:853–862. <https://doi.org/10.1038/ng1598>
- Wang W et al (2015) Ribosomal proteins and human diseases: pathogenesis, molecular mechanisms, and therapeutic implications. *Med Res Rev* 35:225–285. <https://doi.org/10.1002/med.21327>
- Global tuberculosis report 2023. (2023). WHO. <https://www.who.int/teams/global-tuberculosis-programme/tb-reports>. December 25, 2023
- Wilson GG (1992) Amino acid sequence arrangements of DNA-methyltransferases. *Methods Enzymol* 216:259–279. [https://doi.org/10.1016/0076-6879\(92\)16026-g](https://doi.org/10.1016/0076-6879(92)16026-g)
- Xu G, Wang J, Gao GF, Liu CH (2014) Insights into battles between *Mycobacterium tuberculosis* and macrophages. *Protein Cell* 5:728–736. <https://doi.org/10.1007/s13238-014-0077-5>
- Yoder JA, Soman NS, Verdine GL, Bestor TH (1997) DNA (cytosine-5)-methyltransferases in mouse cells and tissues. studies with a mechanism-based probe. *J Mol Biol* 270:385–395. <https://doi.org/10.1006/jmbi.1997.1125>
- Zhang Y et al (2018) The signature of liver cancer in immune cells DNA methylation. *Clin Epigenet* 10:8. <https://doi.org/10.1186/s13148-017-0436-1>
- Zhang J, Yang C, Wu C, Cui W, Wang L (2020) DNA Methyltransferases in cancer: biology, paradox, aberrations, and targeted therapy. *Cancers (basel)*. <https://doi.org/10.3390/cancers12082123>
- Zheng L et al (2016) Unraveling methylation changes of host macrophages in *Mycobacterium tuberculosis* infection. *Tuberculosis (edinb)* 98:139–148. <https://doi.org/10.1016/j.tube.2016.03.003>
- Zhu L et al (2016) Precision methylome characterization of *Mycobacterium tuberculosis* complex (MTBC) using PacBio single-molecule real-time (SMRT) technology. *Nucleic Acids Res* 44:730–743. <https://doi.org/10.1093/nar/gkv1498>

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