Epigenetic-Mediated Antimicrobial Resistance

Subjects: Infectious Diseases

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Antimicrobial resistance (AMR) mechanisms include the horizontal and vertical transfer of resistance genes, gene mutations affecting antibiotic targets, drug influx/efflux strategies, or antibiotic inactivation. Among the common and severely affecting pathogens attributed to AMR development include *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis*, *Helicobacter pylori*, and *Pseudomonas aeruginosa*, but there are many more.

antibiotic resistance epigenetic changes DNA methylation nucleoid-associated proteins

1. Eukaryotic Epigenetic Mechanisms

1.1. DNA Methylation

DNA methylation is a fundamental DNA modification process that occurs mainly at the C5 position of cytosine residues (5mC) on DNA nucleotides and predominantly targets the CpG islands (CGIs). Of the whole genome, CGIs existing in a gene promoter region are most commonly subject to dynamic methylation modifications and gene regulation. DNA methyltransferases (DNMTs) are known as a family of enzymes that catalyze the process of DNA methylation, leading to gene silencing [1]. Additionally, a family of iron-dependent oxygenases—the ten-eleven translocation proteins (TETs)—function to remove the methyl group from the cytosine of the methylated DNA [2].

1.2. Histone Modifications

DNA is wrapped around core histone proteins, and these globular proteins have flexible tails protruding out from the nucleosome. Histone protein tails are subject to various post-translational covalent modifications, such as methylation, acetylation, and phosphorylation [3]. Histone acetyltransferases and deacetylases regulate the histone acetylation system. Histone deacetylation is usually associated with closed chromatin conformation and suppressing gene expression, whereas its acetylation will cause open chromatin conformation, increasing gene transcription [4]. On the other hand, histone methylation via arginine or lysine methyltransferases can facilitate or inhibit gene expression by regulating the DNA accessibility of transcription factors, gene silencing by blocking transcription, or gene overexpression by enabling the binding of transcription factors [5].

1.3. ncRNAs

There are various types of ncRNAs: The housekeeping ncRNAs include transfer RNA (tRNA), ribosomal RNA (rRNA), and small nuclear RNAs (snRNAs), while the regulatory ncRNAs include miRNA and lncRNA [6]. Multiple mRNAs can be targeted by miRNAs binding with the 3′-untranslated regions of mRNAs, leading to inhibition of protein expression. Likewise, lncRNAs modulate chromatin-modifying complexes or directly interact with transcription factors to suppress translation [7]. In recent years, it has been shown that these ncRNAs play significant roles in epigenetic modification by targeting specific gene sequences and transposons, where they exert upregulation or silencing of the gene expression to control cell differentiation [8][9].

2. Overview of Bacterial Epigenetics

2.1. Bacterial DNA Methylation

Bacterial DNA methylation has been studied extensively (**Table 1**) [10]. The DNMTs present in bacteria are more commonly referred to as Mtases that are associated with the bacterial genome defense system, i.e., the restriction–modification (R–M) system. Additionally, a different class of Mtases exists without being associated with any endonucleases—the orphan Mtases—which have housekeeping functions. These Mtases transfer methyl groups to adenine and cytosine to specific genome sequences, leaving the unmethylated DNA sequence degraded by the R–M system [10]. In addition, several Mtases in the R–M system have been shown to have functions in phenotypic cell variations via regulation of transcription [11]. R–M systems represent one of the mechanisms by which bacteria protect themselves against exogenous DNA [12]. Mtases associated with the R–M system are abundantly found in the bacterial genome, the best example of which is *H. pylori*, whose genome encodes for more than 50 R–M-system-related Mtases [13].

Table 1. Overview of bacterial epigenetics through DNA and RNA modifications.

Modifications	Types	Enzymatic Systems	Functions	Examples
DNA	Methylation	R–M system	Defense mechanism	*EcoRV*, *CfrBI*
		Orphan Mtases	Adenine and Cytosine methyltransferases cause regulation of cell cycle, DNA repair, and gene expression	*DAM*, *Dcm*, *CcrM*, *YhdJ*, *VchM*
	Phosphorothioation	DNA degradation	Defense mechanism	*dndABCDE*
RNA	Methylation	N^6-methyladenine modifications	ND [1]	ND [1]
	Capping	5′ NAD capping	Prevent RNA degradation	ND [1]

[1] Not determined.

The orphan Mtases are known to regulate bacterial growth by modulating the cell cycle, DNA mismatch repair, and gene expression [14]. These Mtases generally function as processive enzymes and methylate multiple targets by consecutive reactions without releasing their substrate DNA strand [15]. Deoxyadenosine methylase (Dam), found in *E. coli*, is an excellent example of an orphan Mtase that methylates the N^6 position of the adenine residue, explicitly targeting the GATC sequence and playing a pivotal role in mismatch repair [16]. A common Dam-based methylation system involving GATC motifs, and several type-I R–M systems were identified across seven *K. pneumoniae* isolates [17]. Dam-mediated DNA methylation is also essential for regulating the cell cycle, gene expression, and transgenerational phase variation [18]. In addition to *E. coli*, homologs of Dam have been found in several other Gram-negative bacteria such as *Salmonella enterica* and *Vibrio cholera* [19]. Orphan Mtases are also known to methylate cytosine residues in growth-related genes. For instance, Dcm, found in *E. coli*, and VchM, found in *V. cholera*, control the expression of major gene regulators in the stationary growth phase but are not essential for bacterial survival [20]. Other types of well-studied orphan Mtases include Yhdj and CcrM; both of these Mtases methylate adenine residues at different locations and target different DNA sequences. CcrM is mainly reported to target hemimethylated DNA and regulates the bacterial cell cycle, mainly in Alphaproteobacteria [21].

2.2. Bacterial RNA Modifications

In addition to DNA modification, the presence of RNA modifications in bacterial rRNA, tRNA, and mRNA, depends on the bacterial growth cycle [22]. Of those, N6-methyladenosine (m6A) modification and 5′ NAD capping of mRNA have been reported as the most frequent type of modification in a wide range of bacteria, although the functional significance of RNA-modification-based epigenetic changes is unclear [23][24] (**Table 1**).

2.3. Bacterial Histone-like Proteins (HU)

Instead of having a membrane-bound nucleus similar to the nucleus of eukaryotes, bacteria pack their genomes into nucleoids through a series of nucleoid-associated proteins (NAPs) in distinct cytoplasmic regions. Differences in these NAPs are believed to form regions of chromatin, analogous to eukaryotic transcriptionally active heterochromatin and transcriptionally inactive euchromatin in bacteria [25]. Although it was previously claimed that bacteria do not possess histones and that bacterial epigenetics is limited to DNA methylation [10][26], there is now clear evidence that this is not the case. The HU in bacteria is a highly conserved low-molecular-weight nucleoid-associated proteins (NAPs) and is typically the most abundant across the bacterial kingdom, producing as many as 55,000 HU protein copies per cell in *E. coli* [27]. HUs have been called histone-like proteins due to the manner in which they bind DNA, and like eukaryotic histones, some bacteria (e.g., Mycobacterium and Campylobacter) have HUs with a lysine-rich C-terminal tail. The function of HU protein as a DNA-binding transcription factor indicates its influence on important metabolic processes such as initiation of DNA replication, induction of gene expression related to cell division, and stress response [28]. It can also be presumed to be involved in virulence gene expression in the case of pathogenic bacteria. In many bacteria, it has been found that modification of lysine residues by acetylation occurs on lysines within the core or the C-terminal tail that regulates DNA binding, (**Figure**

1) leading to the suggestion of an epigenetic histone-like code operating in bacteria [29]. The first evidence for these histone-like epigenetic changes came from one using *Mycobacterium smegmatis*, in which heritable but semi-stable drug resistance was seen in bacterial subpopulations, which was determined to be due to the HU acetylation state [30]. Some of the enzymes that catalyze the acetylation of HU also acetylate aminoglycoside antibiotics, leading to their inactivation, and are important mediators of antimicrobial resistance (AMR) [31]. HU-like histones usually act as transcription repressors, and in many bacteria, they are involved in the regulation of virulence and survival.

Figure 1. (**A**) Eukaryotic histones (green) have lysine-rich tails that are acetylated by lysine acetyltransferases, and this result in a reduction in affinity of the histone for DNA; (**B**) the histone-like protein (HU) (blue) of Mycobacterium also has a tail that is rich in lysines, which is acetylated by Eis, leading to a reduction in DNA affinity; (**C**) other bacterial HUs do not have tails but are acetylated at other positions to reduce their affinity to DNA.

3. Bacterial Epigenetics Causing Antibiotic Resistance

DNA methylation induced by Mtases by directly modulating the binding of RNA polymerases can cause positive and negative gene expression. The methylation of cytosine is mainly considered repressive and is commonly found

in many pathogens. Such Mtase-mediated repressive feedback in the R–M system prevents methylation of phage DNAs when present inside the host, thus indirectly contributing to the development and promotion of antibiotic resistance [19]. Several lines of evidence, discussed below, support the notion that epigenetic mechanisms regulate the development of antibiotic resistance in bacteria, which are not fully explained by genetic changes alone.

The bacteria growing in subinhibitory concentrations of the antibiotics are known to develop adaptive resistance due to epigenetic changes. Thus, shifting the same bacteria to antibiotic-free media or exposure to a different type of antibiotic reverses the resistance effect. Therefore, the rapidity and reversible nature of such context-dependent AMR can only be explained by the appearance of epigenetic tags on the bacterial genome and not by genetic mutations [32]. However, only a few evolutionary and gene-knockout have identified epigenetic changes responsible for the development of adaptive resistance, but the role of Mtase-mediated epigenetic tagging of gene promoters influencing the binding of RNA polymerase might be the critical factor in the regulation of AMR-related gene expressions. For instance, in *E. coli*, Dcm-mediated DNA methylation induces the silencing of many genes encoding for ribosomal proteins [20].

Phase variation is a phenomenon where the bacteria can reversibly switch on or switch off specific genes to evade antibiotic effects. One way bacteria modulate the genes related to phase variation is via DNA hypermethylation or hypomethylation. Several Mtases exhibiting the function of phase-variable mediators have been found in bacteria. For instance, the expressions of LPS O-antigen in *S. enterica* and pap operon in *E. coli*, providing resistance via phase variation, are controlled by DNA methylation [33][34]. In *S. pneumoniae*, genetic rearrangement due to random gene switching leads to whole-genome methylation changes and phenotypic phase variation [35]. In *N. meningitidis*, adenine Mtases (ModA11, and ModA112) are known to increase susceptibility to certain antibiotics, which is strangely an evolutionary disadvantage. Nevertheless, the absence of these Mod proteins will increase the chances of bacterial survival [36]. Moreover, various Mtases demonstrating phase-variable expression have been discovered in *H. pylori* [37] and *Haemophilus influenzae* [38], supporting the role of epigenetic-mediated phase variation and development of AMR in these bacteria.

Phenotypic heterogeneity of bacterial population in a changing antibiotic milieu has been shown to induce heteroresistance and bistability, i.e., the appearance of two distinct bacterial subpopulations—the persister bacteria and the sensitive bacteria [39]. Persistent bacterial subpopulations can survive antibiotic treatment, but their growth will be slower or cell-cycle arrest will occur; however, after antibiotic withdrawal, these bacteria can relapse and cause reinfection. Several genetic-based mechanisms cause the survival of persistent bacteria, but recently, epigenetic inheritance has been reported as a potential contributor to the development of such phenotypes [40]. The appearance of heterogeneity and AMR phenotypes leading to recurrent infections has been reported in both Gram-negative and Gram-positive bacteria [41].

The transfer of antibiotic resistance genes in plasmids is known as plasmid-mediated resistance (PMR). This process occurs either via conjugation, with the help of bacteriophage viruses, or when some bacteria can pick up naked plasmids from the environment, and then those plasmids can be transferred between bacteria within the same species or between species. Plasmids frequently include several antibiotic resistance genes, which

contribute to MDR's spread. Antibiotic resistance mediated by MDR plasmids significantly limits treatment choices for bacterial infections, particularly in critically ill patients [42]. A rich variety of plasmids that can harbor numerous virulence factors and resistance genes exists in *K. pneumoniae*, which is the causative agent of serious community- and hospital-acquired infections [43]. Furthermore, there is also a potential epigenetic role of phage-encoded Mtases in AMR development. A vast portion (~20%) of the bacterial genome consists of genes that encode Mtases, which are incorporated into their genome via bacteriophages [44]. Such amalgamation of phage DNAs and bacterial genomes enhances the capability of bacteria to infect several different hosts. More than 800 different types of orphan Mtases were found to be encoded via bacteriophage DNA. For instance, the adenine methyltransferases encoded via phage DNA will methylate a specific DNA sequence, leading to packaging and protection of bacterial DNA from host restriction endonucleases and increasing bacterial survival [45]. However, further insights are needed to understand its application in antibiotic resistance.

Lastly, in several species of bacteria, epigenetic processes contribute to developing AMR by regulating the genes not directly related to antibiotic resistance. For example, resistant strains of *M. tuberculosis* treated with 4-aminosalicylic acid showed differential methylation profiles in thousands of genes, mainly related to the ATP-binding cassette transporter proteins, ribosomal biogenesis pathway, and nitrogen metabolism pathway [46][47]. Integration of transcriptomic and epigenomic analysis in bacteria surviving under antibiotic stress can identify novel genes as potential targets and valuable assets to understanding indirect, epigenetic-mediated regulation of AMR. Nonetheless, our understanding of bacterial epigenetics and its role in antibiotic resistance development is still not fully understood. Moreover, pathogen-mediated host epigenome remodeling can also likely facilitate bacterial survival.

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