Deciphering the Role of Epigenetic Reprogramming in Host-Pathogen Interactions

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Abstract

The success of a pathogen within the host depends on various extrinsic factors that work in a synergistic mechanism to promote pathogenesis. One such factor is driven by the changes observed within the host genome, providing survival and establishment of pathogens inside the host. Pathogens are also known for establishing their intracellular niche within the host by mimicking the host enzymes and immune system for survival. Understanding the strategies used by pathogens to intervene in host genetic machinery for pathogenesis is important for creating successful targets and personalized drugs to counterbalance their effects. Accumulation of omics data and simultaneous development of bioinformatics analysis tools have allowed researchers to understand the interplay between prokaryotic and eukaryotic cells through the multi-omics approach. This permits a better understanding of diseases associated with host-parasite interactions and subsequent development of personalized medicines as therapeutics.

Keywords

Epigenetic modification · DNA methylation · Host signaling pathways · Pathogenic plasticity · Omics technologies · Next generation sequencing · Third generation sequencing

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3.1 Introduction

Pathoepigenetics is an emerging field of microbiology which deals with the epigenetic changes involved in host-pathogen interactions that are vital for the survival and multiplication of pathogens to induce infection within the host. More than 1400 species of human pathogens including viruses, bacteria, protozoans, and helminths have been observed. In order to thrive, they have been evolving along with humans, evading the innate and adaptive immune responses, thereby conquering their host. Understanding the molecular mechanisms involved in epigenetic changes triggered by pathogens is important to demonstrate the signaling pathways affected during infection. In order to beat the devastating infectious diseases, humans have been coevolving with pathogens by altering their genome to co-adapt. The most significant evolutionary machinery consists of a major histocompatibility complex (MHC), which shows diversity within individuals and contains the memory of past infections. Innate and adaptive immune systems collaborate to counterbalance the effects of pathogens. In order to establish themselves, the infectious agents aim to attack the host's defense system. Several bacteria and viruses aim to alter the epigenetic machinery of the host. They have been shown to initiate reprogramming of the innate immune cells. The pathogenic effector molecules modulate histone and protein deacetylation to promote regulatory T cell (Treg) [1]. *Clostridium perfringens* and *Streptococcus pneumoniae* have been shown to secret toxins, namely, perfringolysin and pneumolysin, respectively, which lead to phosphorylation of H3S10. *Listeria monocytogenes* have been shown to induce H3S10 phosphorylation and deacetylation of H3 and H4 histones, thereby altering the chromatin for pathogenesis. Other bacteria have been shown to spread their virulence by modulating HDAC1 family proteins which promote epigenetic tolerance against these microbes [2–7]. The potential role of microbial infections in allergic diseases and autoimmune diseases has also been linked to the modulation of epigenetic factors through altering mucosal surfaces and counterbalancing the innate defense system of the host [8]. Highlighting the potential virulence determinants that epigenetically modulate the host genome will provide an understanding for the development of therapeutics to evade the infection. The dynamic nature of environment-driven epigenetic plasticity has enabled the host and pathogen to find new strategies for the survival of the fittest.

3.1.1 The Epigenetic Code

While the human genome sequence has transformed our understanding of human biology, it is not just the sequence of our DNA that matters, but how we use it and how are things executed within a cellular machinery. Why are some genes activated in certain cell types while others are silenced? Which factors work in synergy to regulate these differentially expressed genes? What properties differentiate a nerve cell from a smooth muscle cell? The key to this is epigenetics. Epigenetic changes are heritable through cell divisions and reversible and hold the potential to be

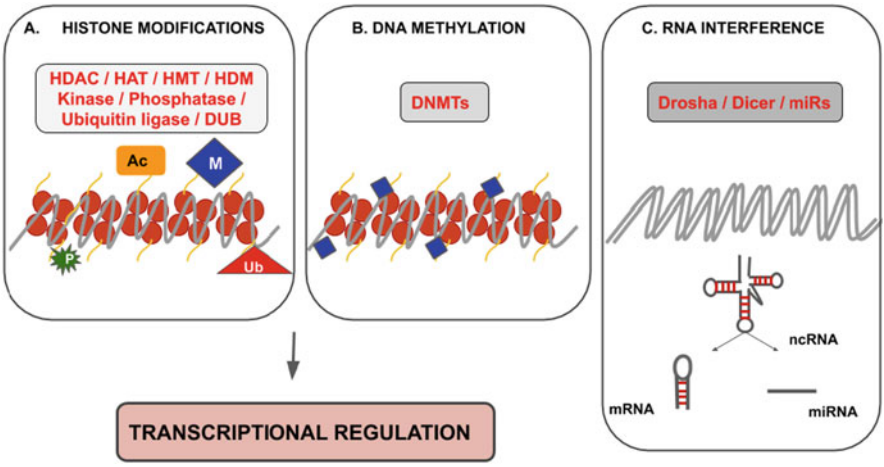

Fig. 3.1 Epigenetic modifications are illustrated here which lead to chromatin remodeling into active or inactive states. (**a**) DNA is wrapped around nucleosomes which are made of histone proteins which are prone to epigenetic alterations. Histone modifications include acetylation/deacetylation by HAT and HDAC, histone methylation/demethylation by HMT, and HDM and histone phosphorylation/dephosphorylation by kinases and phosphatases, respectively, and ubiquitination by ubiquitin ligase which adds ubiquitin to histones and deubiquitination by DUBs. (**b**) DNA methylation includes the addition of methyl groups by DNMTs which leads to transcriptional repression or silencing. (**c**) Epigenetic modifications through RNA interference by cleavage of ncRNAs into mRNAs and miRNAs. These miRNAs sit on the 3′ end of UTRs of mRNAs and thus prevent translation. Ac, acetyl; DUB, deubiquitinases; HDAC, histone deacetylases; HAT, histone acetyltransferases; HMT, histone methyltransferases; HDM, histone demethylases; DNMTs, DNA methyltransferases; M, methyl; miRNA, microRNA; mRNA, messenger RNA; ncRNAs, noncoding RNAs; P, phosphate; Ub, ubiquitin

manipulated therapeutically. These modifications are sensitive to the environment. Epigenetics is the study of factors associated with behavioral and environmentally induced heritable changes within the gene expression that arise from chemical modifications of DNA or histone proteins. These changes are known to alter the phenotype of an organism without changing the genotype.

Molecular analysis shows that epigenetic changes comprise covalent modifications like DNA and histone methylation, phosphorylation, ubiquitination, SUMOylation, ADP ribosylation, citrullination, and acetylation [9, 10]. Eukaryotic DNA is tightly wrapped around the histone proteins. Majorly studied eukaryotic epigenetic mechanisms comprise methylation of cytosine residues in DNA and histone modifications that regulate nucleosome stability. Posttranslational modifications (PMTs) like histone methylation/demethylation and acetylation/deacetylation result in changes associated with the switching on and off of genes. These chromatin modifications are modulated by enzymes known as "writers," like certain kinases, histone acetyltransferases (HATs), and histone methyltransferases (HMTs), and "erasers" like phosphatases, histone deacetylases (HDACs), and histone demethylases (HDMs) [11] (Fig. 3.1). Bacteria, on the other hand, lacks

histones; thus, the major epigenetic modifications include adenine and cytosine methylation which regulates gene expression and consists of a restriction-modification system which protects bacterial DNA from cleavage [12].

3.1.2 Epigenetic Reprogramming Driven by Extrinsic Factors

Once thought to be simply heritable, epigenetic changes are those extrinsic changes which are now considered to modulate the intrinsic environment throughout the organism's lifespan during cellular differentiation. These extrinsic changes include physical environmental stresses, lifestyle, nutritional factors, environmental toxins, and pharmacological treatments an organism undergoes during its lifetime. The prevailing environmental conditions can modulate the genetic expression of a trait through epigenetic alterations providing plasticity to the organism for adapting to the environment [13]. Thus, epigenetic changes ensure the induction of alternative phenotypes without an actual change in the genotype of the organism. Understanding the interactions between these environmental factors and their impact on the epigenome can help us predict the healthy or disease-associated phenotypes of the organism [14]. The environment-induced epigenetic changes are also dependent on the titer of infection or bacterial load and the duration of infection [15]. For this, the bacteria must establish itself in the microenvironment of the host by evading the host defense mechanisms. The higher the bacterial load and duration of infection within the host, the greater will be the epigenetic changes.

3.1.2.1 DNA Methylation

It is an epigenetic change marked by the addition of a methyl group to bases in the DNA sequence. The most frequently studied methylation is of the C5 position on cytosine bases using DNA methyltransferases (DNMTs) as writers [16, 17]. CpG methylation is the most dominant form of methylation in eukaryotes which can suppress transcription by blocking DNA binding by transcription factors, while in bacteria, methylation of the adenine residues is the main epigenetic signal. Immunoprecipitation and bisulfite-based techniques can be used together with microarrays or next-generation sequencing to decipher the genomic regions that are epigenetically modified. Recently, changes in DNA methylation induced by *E. coli* were observed in porcine cells where DNA methylation was shown to be majorly affected in immune response genes [18]. *Helicobacter pylori* infection can cause DNA methylation in the human gastric mucosa within genes associated with gastric cancer [19–21]. Within the uroepithelial cells, *E. coli* infection results in the upregulation of DNMT expression which induces CpG methylation which enables pathogen persistence within the host [22].

3.1.2.2 Histone Modification

Posttranslational histone remodeling can be achieved in different ways like histone acetylation, methylation, phosphorylation, and ubiquitination. Acetylation is catalyzed by histone acetyltransferase enzymes (HATs) which add an acetyl group

to the positively charged lysine amino acids within the histone tails, thus masking the positive charge. Transcriptionally permissive modifications include H3/H4 histone acetylation at the ε-amino group of lysine residues [23]. In contrast, deacetylation of histones is carried out by HDACs (histone deacetylase enzymes) and correlates with CpG methylation and inactive state of chromatin, thereby repressing transcription. HDACs are also regulated by phosphorylation, acetylation, and SUMOylation. Histone methylation includes modifications like H3K9me and H3K27me which can be related to chromatin repression [24], whereas H3K4me3, H3K36me3, H3S10p, and H3K14ac modifications are related with chromatin activation [25]. Furthermore, methylation can occur on ε-amino groups of arginine or lysine amino acids catalyzed by histone methyltransferases, but without any change in the charge of amino acids. This modification can be associated with both active and repressive gene transcription [26]. RV1988, a methyltransferase secreted by *M. tuberculosis*, methylates histone H3 at residue R42, promoting gene activation [27]. Mass spectrometry and genomics-based techniques such as ChIP-seq and ChIP-chip can be applied to detect specific regions of the genome associated with histone modifications. Bacterial histone acetylation/deacetylation and phosphorylation/dephosphorylation are involved in the alteration of microbe-associated molecular patterns and virulence factors involved in host-bacteria interactions. Histone methylation is the major histone modification targeted by bacteria [28]. SET domain proteins from various bacteria, like *Burkholderia thailandensis* and *Bacillus anthracis*, have been shown to cause histone methylation for transcriptional modification in the host [29].

3.1.2.3 RNA-Based Silencing

Gene regulation can also be achieved by antisense transcripts, by noncoding RNAs, or through RNA interference. RNA-based silencing alters the gene expression by triggering histone modifications or DNA methylation resulting in heterochromatin formation [25]. Within the nucleus, different long noncoding RNAs regulate the epigenetic status of various protein-coding genes, modifying gene transcription by recruiting chromatin remodeling complexes [30]. Long ncRNAs and sRNAs have been reported to participate in various regulatory processes involving chromatin or transcriptional regulation, nuclear architecture, and RNA processing [31–33]. lncRNAs alter the epigenetic processes by remodeling chromatin structure, while miRNAs are known to regulate DNMT expression in somatic cells and during embryonic development [34]. IsrM, one of the sRNAs of *Salmonella*, has been shown to promote bacterial invasion in hosts [35]. Recently, Gao et al. identified the survival strategy of bacteria *Edwardsiella tarda* within the intestine of humans by modulating sRNAs for establishment in hosts [36].

3.1.3 The Epigenetic Bridge of Survival: How Pathogens Change the Epigenetic Signals to Modulate Gene Transcription and Translation

The epigenetics of host-pathogen interactions aims to understand the dynamic and plastic nature of pathogenicity which directly links to the successful alteration of the host environment for survival and transmission of pathogens. Pathogens conquer the epigenetic signaling by altering the epigenetic modifications of genes associated with virulence processes, which allows their colonization, replication, and dissemination within the host. Bacteria secrete effector molecules like nucleomodulins [28] which enter the host nucleus and hijack the epigenetic machinery by manipulating the epigenetic factors, sRNAs, ncRNAs, and mRNAs [37] (Fig. 3.2).

3.1.3.1 The Bacterial Epigenome

Bacteria also utilize epigenetic modifications for various cellular functions like DNA replication, DNA repair, bacteriophage packaging, transposition, chromosomal segregation, transcriptional regulation, and interestingly, alteration of host cellular environment for pathogenicity. Adenine methylation is one of the extensively studied epigenetic modifications in bacteria which is reported to be regulated by DNA adenine methyltransferase (Dam) in *E. coli* and *Gammaproteobacteria* [38] while cell cycle-regulated methyltransferase (CcrM) has been studied in *Alphaproteobacteria* [39, 40]. DNA adenine methylation was found to be vital for *Salmonella* species [41, 42]. Restriction-modification systems in bacteria are known to have their own DNA methyltransferases which protect self-DNA from degradation after cleavage by restriction-modification enzymes [43]. Bacteria are shown to undergo a tremendous amount of phase variation which involves random and reversible switching of gene expression resulting in a wide variety of phenotypic cell variants [44, 45] known as phasevarions [46]. These phasevarions exhibit a heterogeneous mixed expression state with the gene either in the "active" or "repressed" state. This equips bacteria for immune evasion by providing a better resistance strategy for colonization inside the host environment and escaping membrane-specific vaccines. Such bacteria are categorized under the human-adapted pathogens, most commonly studied in *E. coli*, *Haemophilus influenzae*, *Helicobacter pylori*, and *Salmonella* species [47, 48]. Such changes are mediated by methyltransferases of the restriction-modification system and Dam. An outer membrane protein antigen 43 (Ag43), encoded by the Agn43 gene in *Escherichia coli*, is important for biofilms and infection. It is controlled by phase variation mediated by two proteins, Dam and the oxidative stress regulator OxyR. The GATC sequences of the promoter region of Agn43 gene overlap with the OxyR binding site. The binding of OxyR to this regulatory region of Agn43 leads to transcription repression of Agn43. However, Dam methylation of GATC sequences results in the transcriptional activation of Agn43 by preventing OxyR binding [49]. Phase variation has been known to cause immune evasion in a wide variety of bacteria like *Streptococcus pneumoniae*, *Clostridioides difficile*, *Vibrio*, and *Haemophilus* [50–53].

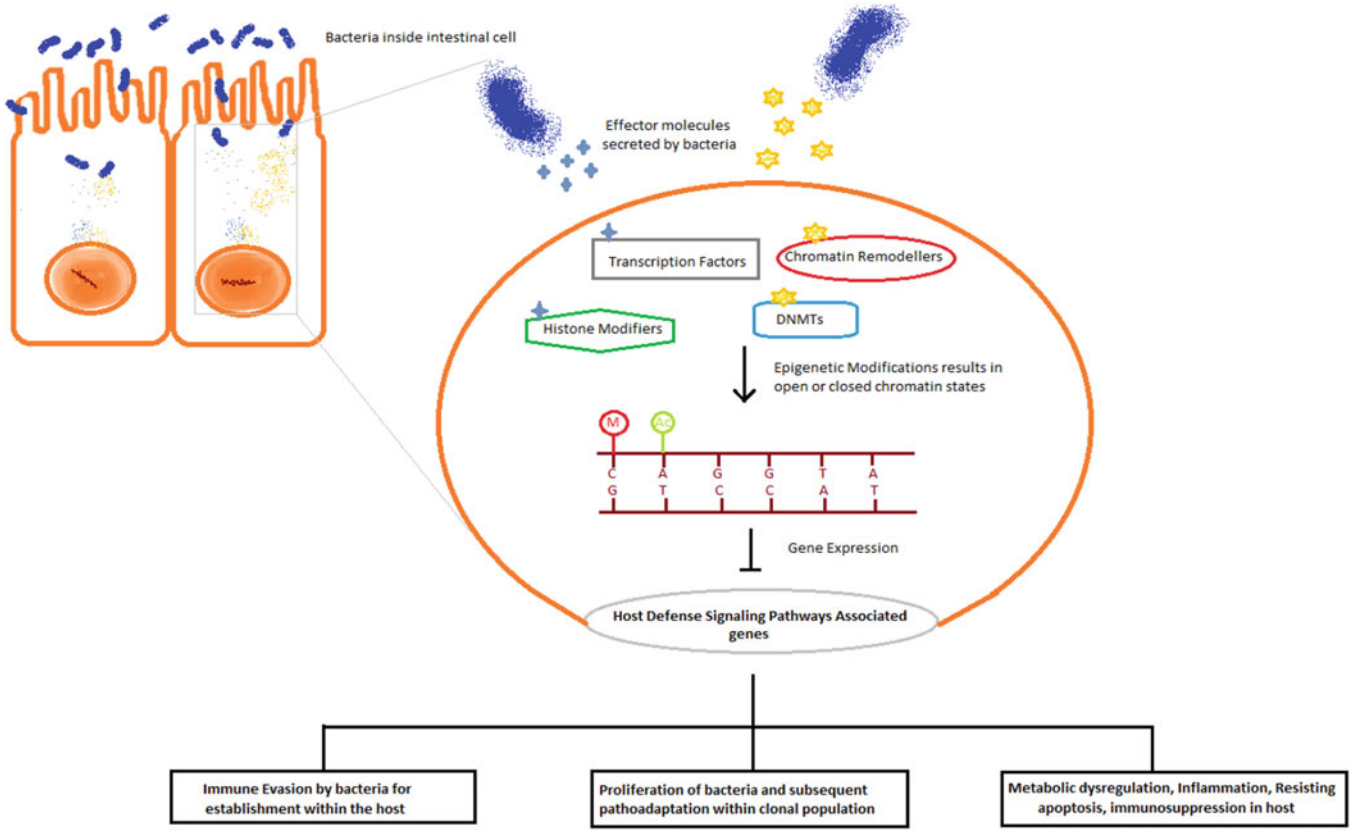

Fig. 3.2 The epigenetic modifications of bacteria within human intestinal cells. Bacteria secrete effector molecules like nucleomodulins which bind to transcription factors, chromatin remodelers, histone modifiers, DNMTs, etc. to regulate gene expression. Silencing or activation of genes associated with host defense pathways enable successful niche establishment, proliferation, adaptation, and survival of bacteria within the host

3.1.3.2 Pathogenic Plasticity

Bacterial genome plasticity contributes in shaping host-pathogen interactions for the colonization, invasion, survival, multiplication, and transmission of bacteria within the host. The challenges faced by pathogens within the diverse host environment elicit adaptive changes and mutations which can be observed morphologically and developmentally within the pathogens, thus rendering them protection from host defenses and therapeutic interventions. In order to facilitate survival in the host, bacteria acquired various strategies to terminate host cellular responses by altering host signaling pathways [54], targeting chromatin regulation, and modulating epigenetic marks. Bacteria encode certain effector molecules that modify host epigenetic machinery [55]. Protist *Plasmodium* has adapted to the host environment by exhibiting erythrocytic and hepatocytic stages which meet the pathogen's developmental requirements and enable it to survive longer within the host. These stages encode for genetic diversity and plasticity within the clonal population of pathogens. These patho-adaptive changes contribute to the fitness of pathogens. Similarly, bacteria undergo selective pressures within the host which allow genetic changes contributing to pathogenic plasticity. Within the same species, bacterial strains show variations in symptomatic and long-lasting asymptomatic cycles of infection. Pathogenic bacteria like *E. coli*, *M. tuberculosis*, and *S. typhi* can be asymptomatically carried as a symbiont in hosts without showing any symptoms of infection [56]. They escape detection by hiding inside the macrophages within granulomas [57].

3.1.4 Host Signaling Pathways Altered for Pathogenicity

The effects of host-pathogen interaction revolve around alteration of host signaling cascades which are important for bacterial colonization in the host niche. For successful establishment, bacteria need to modify their defense system for evasion from the host, weaken the host immune system, and alter the host cellular machinery by mimicking host-like factors [54]. Certain bacteria have been shown to modify chromatin factors resulting in altered transcriptional regulation. In order to weaken the host defense system, bacteria aim to target the immune-specific signaling pathways. This works by altering the state of chromatin resulting in the conversion of euchromatin and heterochromatin or vice versa. Bacteria are involved in alteration of host MAPK, PI3K, and NF-κB signaling cascades leading to downstream activation of kinases like AKT, IKK-α, and MSK which are involved in histone H3S10 phosphorylation and acetylation of H3K14 and H4K8 at the promoter regions of pro-inflammatory genes like IL-8 due to transcriptional repression [58, 59]. This results in the suppression of host inflammatory response against the invading bacteria [60, 61]. Gram-negative bacteria like *Shigella flexneri* have been shown to inhibit MAPK pathway and subsequent blocking of pro-inflammatory genes [62, 63]. The production of metabolites by bacteria leads to inhibition of chromatin-modifying enzymes in the host. One such metabolite, butyric acid, acts as an inhibitor of HDACs [64]. Also, certain bacteria like *Anaplasma*

phagocytophilum, *Ehrlichia*, and *Coxiella* have been shown to produce Ank-containing proteins which bind to the host nuclear chromatin. The motifs of bacterial Ank exhibit evolutionary homologies with eukaryotic counterparts. These result in altering protein-protein interaction and transcriptional regulation in the host imparting survival to the pathogen [65–67]. Differentially methylated CpGs in *E. coli*-infected porcine cells are composed of pro-inflammatory molecules like PAX5, AP4, IRF2, XBP1, and CREB with a significant reduction in DNA methyltransferases (DNMTs) which control the epigenetic modifications of the host [19].

3.2 Omics Technologies to Investigate Host-Pathogen Interactions

Traditional methods for diagnosing bacterial infections are composed of sensitive microbial cultures and isolation, followed by serological, immunological, and biochemical detection [68]. However, due to differences between in vivo host environments and in vitro cultures of bacteria, the host-pathogen interaction studies were incomplete. Also, detection of genetic, epigenetic, and metabolic differences initiated by pathogens was not possible through traditional culture and serological diagnosis [69, 70]. Molecular detection methods included real-time polymerase chain reaction, antimicrobial susceptibility testing, mass spectrometry (MS)-based methods [71–73], and immunoassays which are still considered gold standard methods for the identification of bacterial infections. However, due to insensitivity in the detection of certain species and strains, the diagnosis remains limited. These conventional diagnostic methods and molecular characterization methods have been successful in the identification of infections and controlling pandemics, but they are very laborious and time-consuming with poor resolution and specificity [74].

With the spread of infectious agents and increment in death rates as a result of bacterial infections, modernized technologies have gained popularity in high-throughput detection of these causative agents [75]. An advent of sequencing technologies have allowed researchers to understand the in vivo dynamics of pathogenesis [76]. With the revolution in high-throughput sequencing, whole genome sequencing has become a routine tool for clinical microbiology [77, 78]. The challenges provided by outbreaks of drug-resistant bacteria pose huge threats to the medical community. Therefore, it is important to understand the transmission, colonization, and establishment of pathogens within the host through genotypic tools. Due to greater diversity, strain-specific bacteria could not be identified through clinical diagnostic tests and first-generation sequencing methods. A more advanced second-generation sequencing platform permits bacterial genomes sequencing within hours. Whole genome sequencing and comparative genomics of *Escherichia coli* isolates showing diverse toxicity have been used to access the virulence of different strains. This data has been combined with epidemiological and phenotypic analysis to analyze the risk prediction during outbreaks. This was used to predict the marker genes for virulence of the pathogen using

GWAS studies [79, 80]. Sequencing technologies are rapidly improving. Third-generation sequencing platforms provide additional information with longer reads and accurate prediction of methylation sites within less time. This chapter mainly focuses on the methods used to predict epigenetic changes in bacterial infections (Table 3.1).

3.2.1 Epigenomic Techniques to Study Host-Pathogen Interactions

Technical challenges in studying the impact of bacterial load and associated changes in the intracellular environment of the host have been replaced with omics technologies. Over the last two decades, several assays have been designed for assessing the epigenetic changes. These are described in the following sections.

3.2.1.1 ChIP Assay

Chromatin immunoprecipitation assay monitors the epigenetic changes and transcriptional regulation associated with DNA-protein interactions [97]. ChIP assays use formaldehyde to crosslink DNA sequences and DNA-binding proteins in the form of complexes within the bacterial cells. This is followed by fragmentation of bacterial DNA and targeted immunoprecipitation of the resulting complexes. Being semiquantitative, ChIP assays have been used in combination with real-time polymerase chain reaction (ChIP-qPCR) to obtain a quantitative measurement of the amount of DNA of interest bound to protein. This can be validated with other transcriptional profiling methods like deep sequencing, qRT-PCR, and DNA microarrays for transcript-level studies. ChIP assays have been used to study gene regulation in the intracellular pathogens. Since intracellular bacteria have been known to regulate host gene expression by modifying chromatin and associated histone proteins, ChIP assays have been extensively used to study gut microbiota population in *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhimurium* infections [98–102]. These gut-on-a-chip systems have been used to understand the symbiotic associations between the human gut and microbiota [103]. ChIP microarray was used in combination with luciferase reporter assay for studying the molecular basis of gastric tumorigenesis associated with *H. pylori* infection. Methylation profiling identified hypermethylation in tumor suppressor FOXD3 promoter in mice and humans during *H. pylori* infections [104].

3.2.1.2 DNA Methylation Analysis

Traditional methods to identify DNA methylome used bisulfite treatment of DNA to determine methylation patterns in cells known as bisulfite sequencing (BS). This technique was considered a "gold standard" technology since it was extensively used to identify differentially methylated regions on CpG islands before the onset of NGS era, but it cannot be used to detect methylated adenine residues which are commonly altered in bacterial infections. Reduced representation bisulfite sequencing (RRBS) is a modification of bisulfite sequencing which combines BS with restriction enzymes to measure methylation levels on CpG sequences. RRBS in combination

Table 3.1 Omics technologies to study host-pathogen interactions

Type of approach	Description and use	Year	References
Serological diagnosis			
Flocculation tests	Involves flocculation or precipitation of antigen-antibody interactions	1876	[81]
Enzyme-linked immunosorbent assays (ELISAs)	Used to detect the presence or absence of microbial antigens using fluorescent or chemiluminescent or colorimetric signal readouts and quantify the signal	1971	[82]
Chemiluminescence immunoassays	Used to detect light signals which are emitted through the chemical reaction between probes or enzymes that are bound to specific antibodies	1995	[83]
MS proteomics	Involves isolation of bacterial pathogens from host cells followed by enzymatic digestion of proteins and resulting peptides are used for quantification and analysis with mass-spectrometry	1898	[84]
Chromatin immunoprecipitation studies			
N-ChIP	Uses unfixed native chromatin which is digested by nuclease yielding efficient immunoprecipitation of DNA. It is used to study tightly bound histone proteins	2003	[85]
X-ChIP	Uses fixed chromatin which is fragmented by sonication and is mainly used to study nonhistone proteins	2000	[86]
ChIP Cloning	Based on cloning and sequencing of immunoprecipitated DNA obtained from the standard ChIP method	2002	[87]
ChIP-qPCR	ChIP is combined with qPCR to quantify the amount of DNA bound to protein	Early 2000s	[88]
ChIP-CpG microarray	It is used to target ChIP sequencing of CpG islands where transcription factors binding to promoters can be detected. This method uses a combination of ChIP-PCR and microarray to study histone modifications	2003	[89]
DNA methylation analysis (NGS)			
Bisulfite sequencing (BS)	Utilizes bisulfite treatment of DNA to decipher methylation patterns within the cells	1992	[90]
Reduced representation bisulfite sequencing (RRBS)	Combines BS with restriction enzymes to measure methylation levels on CpG sequences	2005	[91]
MeDIP sequencing	Uses antibodies for the enrichment of differentially methylated regions	2005	[92]
Oxidative BS or oxBS-Seq	A modification of BS which can differentiate between 5-methylcytosine and 5-hydroxymethylcytosine after oxidizing DNA to form 5-formylcytosine	2012	[93]

(continued)

Table 3.1 (continued)

Type of approach	Description and use	Year	References
TAB-seq	Modification of BS which glucosylates 5-hydroxymethylcytosine and utilizes TET enzymes to convert 5-methylcytosine to 5-formylcytosine	2012	[94]
Third generation sequencing			
Single-molecule real-time (SMRT) DNA sequencing	To identify altered methyltransferases in bacterial infections along with positions of DNA modifications and has been successfully used in sequencing bacterial methylomes	2009	[95]
Nanopore MinION sequencing	It is used to identify methylated adenine and cytosine residues in bacterial DNA	2014	[96]

with RNA-seq transcriptomic profiling has been used to identify the differentially methylated regions in *Mycobacterium bovis*-infected cattle where epigenetic changes as a result of infection created dysfunctional CD4(+) T lymphocytes which were unable to clear *Mycobacterium* infection [105]. MBD-seq or methylated-CpG binding protein and MeDIP sequencing or methylated DNA immunoprecipitation reaction utilize antibodies for the enrichment of differentially methylated regions with better sensitivity in low CpG dense regions. Integrated MeDIP-ChIP and transcriptome analysis have been used to identify novel methylated signatures in porcine *Escherichia coli* induced diarrhea where changes associated with DNA methylation were observed in immune responses related genes, thus suppressing the host immune system [106]. Whole genome bisulfite sequencing (WGBS) technologies were developed which provided genome coverage at a single-base resolution, but due to higher expenditure, it is not extensively used. Deep sequence coverage of low CpG dense regions was achieved at a cost-effective and more accurate method by methylation capture sequencing or MethylCap-Seq technology. Restriction enzyme-based methods like methyl-sensitive cut counting (MSCC) depend on the restriction enzyme (like Msp1) digestion of CCGG motifs. Other modifications of BS are oxidative BS or oxBS-Seq and TAB-seq which were developed in 2012 since the traditional BS methods could not differentiate between 5-methylcytosine and 5-hydroxymethylcytosine, a TET-mediated modification of methylated cytosine. Ox-BS libraries and TAB-seq or Tet-assisted bisulfite sequencing allow identification of differentially methylated and hydroxymethylated regions at a single-base resolution.

3.2.1.3 Third Generation Methylome Profiling Technologies

Current advances in sequencing technologies allow interpretation of individual DNA molecules and identification of associated base modifications. For an in-depth characterization of the bacterial methylome, the most common third generation platforms include single-molecule real-time (SMRT) DNA sequencing [107] and

Nanopore MinION [108–110] sequencing that allow direct readouts for DNA modifications at a single-base resolution.

Nanopore DNA Sequencing Technology

Nanopore DNA sequencing technology developed by Oxford Nanopore Technologies (ONT) exploits differences in ionic current that occurs when different nucleotide bases pass through genetically modified protein nanopores. Nanopore MinION has been used to characterize bacterial methylomes for the identification of methylated cytosine and adenine residues in the DNA [111]. De novo-based sequencing for Nanopore has not been done so far.

SMRT DNA Sequencing Technology

SMRT DNA sequencing technology was manufactured by Pacific Biosciences Inc. (PacBio), is able to identify altered methyltransferases in bacterial infections along with positions of DNA modifications, and has been successfully used in sequencing bacterial methylomes. The output of SMRT includes simultaneous generation of nucleotide sequence and bacterial DNA methylation signatures (5mC, 4mC, and 6mA) with the relatively high signal-to-noise ratio. SMRT was used to identify methylated adenine residues in *Escherichia coli*-infected cells [107]. SMRT technology has provided deeper insights in understanding phase-variable methyltransferases [112, 113] in various species of bacteria including *Helicobacter pylori* [46, 114], *Haemophilus influenzae* [115], *Neisseria meningitides* [113], and *Campylobacter jejuni* [116].

3.2.1.4 Single-Cell Epigenomics

Investigating the role of single-cell epigenomics has gained popularity, and it is used for characterizing cellular identity, molecular function, and understanding the phenotypes which cannot be predicted solely by the genotype. Epigenetic alterations can be identified as early-stage biomarkers for understanding the pathogenicity of infection and its therapeutics. Most common single-cell methylome assays include reduced-representation bisulfite sequencing (scRRBS), single-cell whole genome Bisulfite sequencing (SC-WGBS), or single-cell bisulfite sequencing (scBS-seq) for the identification of DNA methylation patterns and single-cell chromatin immunoprecipitation sequencing (scChIP-seq) for transcription factor identification and histone modification detection; scDNAse-seq and scATACseq have been used for understanding the chromatin state and scHIC for chromosome conformation capturing.

3.3 Conclusion

A systematic approach towards reduction of pathogenic load and prevention of risks associated with pathogens led to the development of the microbial risk assessment (MRA) tool. Assessing the microbiological load helps in estimating the public health risk by quantifying the extent of spread of a disease or transfer of pathogens

preventing epidemic-like situations. Characterization of the severity of an infectious disease by next generation omics can help in refining our knowledge of the virulence of the pathogen. NGS technologies and high-throughput data analysis have produced innovative technologies for interpreting and understanding complex healthcare attributes. These NGS technologies include RNA-seq, and the expansion of genomics, transcriptomics, metabolomics, and proteomics has enabled us to monitor the individual strategies used by the pathogens for establishment inside the host. Integrating multi-omics approaches with research data has helped us in understanding the host-pathogen interactions. Detection of factors, genes, mimicked enzymes, and signaling components causing the infection through comparative genomics and analysis of these factors as potential biomarkers for the disease can help in the quick prediction and personalized therapeutic development for each strain of pathogen.

3.4 Future Perspectives

Understanding how bacteria mediate multiple levels of cellular and molecular states is fundamental to biomedical research. Multi-omics data integration combines multiple datasets generated by diagnostic tools and sequencing platforms with statistical analysis and correlates this information with biological pathway databases in order to relate the molecular dynamics of a diseased phenotype. These strategies have been in progress with the advent of third-generation sequencing technologies and production of bioinformatics tools to enable high-throughput data generation and analysis. Numerous data repositories have been developed which include Roadmap Epigenomics, Ensembl, Omics Integrator, 3Omics, Panther, String, DAVID, GenExp, Epigenome Atlas, VANTED, ProMeTra, and IntegrOmics. High-dimensional omics data require sophisticated software tools for analysis. Pipelines for analyzing omics data have been advancing along with the data generation. For each dataset, there is dynamicity in the implementation of these pipelines with minor to major changes associated with parameter modifications. Dependency on bioinformatics tools and repositories poses new challenges for advancement in analyzing multi-omics data with a higher resolution. Third generation sequencing methods possess immense potential in uncovering the dynamics of host-pathogen interactions at the molecular, cellular, and tissue-specific level. Most of the DNA methylation aiming at understanding host-pathogen interactions investigated tissue samples. Due to limited biopsy samples, there is a need for noninvasive DNA methylation methods for the detection of epigenetic modifications. One such advancement is observed in single-cell epigenome sequencing technology which provides a basic picture of disease-associated changes in cellular populations infected with pathogens. If used in combination with single-cell transcriptome sequencing, single-cell epigenome sequencing will provide us a better understanding of the dynamics of host-pathogen interactions [117]. Researchers are now investigating cell-free DNA sequencing technologies which harbor body fluids like serum, urine, and plasma for sequencing [118]. Even though Nanopore technology and SMRT need additional improvements, they continue to be promising platforms

for the identification of novel methyltransferases and methylated sites. Third generation methylome studies in collaboration with transcriptome studies and microarray will produce thousands of highly accurate and novel isoforms which will enable us to understand the in vivo dynamics of host-pathogen interactions. Identification of stage-specific biomarkers will allow us to diagnose the infection at earlier stages. Integrating the biomarker information and multi-omics data as a systems biology approach will enable us to unravel the high complexity of the biological system with better delivery of personalized therapeutics or targeted interventional therapies.

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