Chapter 5
Epigenetic Programming by Microbial Pathogens and Impacts on Acute and Chronic Disease

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Abstract Epigenetic programming of the pathogen and the host can have a marked influence on the development and progression of acute and chronic disease. Bacterial pathogenesis may be viewed as a developmental program similar to that of cell differentiation and development in eukaryotes. Bacterial epigenetic programming is imparted by DNA methylation, whereby the virulence traits expressed by a pathogen may depend on the cumulative interactions between the microbe and its environment. Such bacterial "memory" provides a means for adaptation to the varied subsequent microenvironments encountered during the infective process. DNA methylation can affect DNA–protein interactions and resultant gene expression by altering DNA thermodynamic stability and curvature and by methyl-group-mediated steric hindrance. Some of these epigenetic interactions can form heritable DNA methylation patterns in the microbial genome that control gene expression in their progeny cells. Microbes can also stimulate heritable changes in the host epigenome via infection-associated alterations to host epigenetic determinants including DNA methylation, histone modifications, chromatin-associated complexes, and noncoding RNA-mediated silencing. The resultant changes in host chromatin remodeling and gene expression may be localized and/or systemic due to direct microbe-to-host cell communication or via dissemination of microbial-host signaling. Thus, the role of epigenetics in host–microbe interactions may be the nexus of many pathological syndromes even though there may be no apparent direct link between infection and disease, providing the basis for the development of novel therapeutics and diagnostic tests for diseases with epigenomic determinants.

Keywords Epigenetics • DNA methylation • Epigenetic disease • Infectious disease • Epigenetic programming • Epigenome • Bacterial memory • Epigenetic host-microbe interactions • Epigenetic memory

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5.1 Introduction

Deciphering the mechanisms that govern epigenetic programming in the pathogen and the host is crucial to the development of new therapeutic approaches to control acute and chronic disease. For instance, pathogenic *Escherichia coli* utilize heritable DNA methylation patterns to control pili production via a phase variation mechanism, whereby individual cells either express pili (phase-ON) or not (phase-OFF), resulting in periods of attachment and detachment that are critical for progression of an ascending urinary tract infection (Low and Casadesús 2008; Marinus and Casadesús 2009). Microbial infection can also stimulate epigenetic changes in the host epigenome, potentially leading to a variety of human diseases including cancer and autoimmune disorders (Bierne et al. 2012; Dawson and Kouzarides 2012; Elinav et al. 2013; Feinberg and Tycko 2004; Stein 2011). Despite this knowledge, the role of epigenetic modifications on pathogen virulence is poorly understood, and the role of host epigenetic modifications that contribute to, or result from, infectious diseases are only just beginning to be elucidated.

5.2 DNA Methylation

DNA methylation is a fundamental epigenetic process that provides a means to impart additional information to the genomic sequence. In bacteria, DNA methylation occurs at the N^6 position of adenine (6mA) and the C^5 or N^4 positions of cytosine (5mC; 4mC), and these modifications are catalyzed by DNA methyltransferases (Noyer-Weidner and Trautner 1992; Palmer and Marinus 1994; Sánchez-Romero et al. 2015; Wion and Casadesús 2006). Such epigenetic information can alter the timing and targeting of cellular events including transcription, transposition, chromosomal replication, and DNA repair. The most common DNA modification in eukaryotes is 5mC, which is involved in a variety of processes including gene regulation, genomic imprinting, X-chromosome inactivation, and epigenetic memory maintenance (Jones 2012; Jones and Takai 2001; Smith and Meissner 2013). Additionally, 6mA has been recently reported as a possible epigenetic mark in eukaryotes that plays a potential role in transcription and epigenetic inheritance (Luo et al. 2015).

5.2.1 Bacterial Restriction Modification Systems

DNA methylation is a standard means by which restriction-modification (R-M) systems serve to protect bacterial cells from foreign DNA (viruses, transposons, plasmids) (Kobayashi et al. 1999; Meselson et al. 1972; Roberts and Macelis 2001). In most R-M systems, base methylation by a methyltransferase (on adenine or

cytosine) prevents DNA cleavage of host DNA by cognate restriction enzymes. Recent evidence suggests that the role of R-M-associated methyltransferases is not restricted to protecting host genomes as the lack of certain R-M systems alters the gene expression pattern of the cell, suggesting a role in epigenetic control of gene expression (Fang et al. 2012; Furuta et al. 2014; Sánchez-Romero et al. 2015; Vasu and Nagaraja 2013). *E. coli* O104:H4 is a hemolytic uremic syndrome (HUS)-linked outbreak strain that harbors multiple active adenine methyltransferases—some of which are associated with R-M systems (Fang et al. 2012). *E. coli* O104:H4 also contains a lysogenic lambdoid phage, fStx104, which encodes Shiga toxin (the cause of HUS), and an R-M system that engenders both the production of Shiga toxin and alteration of the bacterium's transcriptome. These findings indicate that DNA methyltransferases associated with R-M systems can make considerable contributions to bacterial virulence (discussed further below).

5.2.2 Solitary DNA Methyltransferases

Some bacterial DNA methyltransferases lack cognate restriction enzymes and thus are not part of R-M systems. These solitary methyltransferases play roles in cellular regulatory events including those that control bacterial gene regulation, cell-cycle events, and virulence (Low et al. 2001; Marinus and Casadesús 2009; Reisenauer et al. 1999).

5.2.2.1 Dam Methylase

DNA adenine methylase (Dam) is a solitary methyltransferase of *Gammaproteobacteria* (*e.g.*, *E. coli* and *Salmonella*) that methylates the N^6 position of adenine in the sequence "GATC" of the bacterial genome and plays a role in the timing and targeting of many cellular events by influencing the interactions of regulatory proteins with DNA (Casadesús and Low 2006; Løbner-Olesen et al. 2005; Low and Casadesús 2008; Low et al. 2001; Marinus and Casadesús 2009). DNA adenine methylation can affect DNA–protein interactions at GATC sequences by altering DNA thermodynamic stability and curvature and by methyl-group-mediated steric hindrance (Wion and Casadesús 2006). There are about 130 molecules of Dam per cell in *E. coli*, a level that allows sufficient time for some DNA–protein binding between DNA synthesis and the methylation of GATC sequences within newly synthesized DNA (Boye et al. 1992). Competition between Dam and DNA-binding proteins resulted in the formation of ~35 nonmethylated GATC sequences in the *E. coli* genome (Hale et al. 1994; Ringquist and Smith 1992; Tavazoie and Church 1998; Wang and Church 1992). The actual number of nonmethylated sites at any one time is dependent on bacterial growth rate and growth phase, supporting the hypothesis that the DNA-binding proteins are in

competition with Dam at these sites to control the timing and targeting of cellular regulatory events.

5.2.2.2 Cytosine Methylases

Although the role of cytosine methylases has been generally associated with R-M systems, recent evidence suggests that this view may need to be broadened (Marinus and Casadesús 2009; Sánchez-Romero et al. 2015). DNA cytosine methylase (Dcm) is a solitary methyltransferase of *Gammaproteobacteria* that methylates the internal cytosine in the CCA/TGG motif at the C5 position (5mC) (Bigger et al. 1973; Kahramanoglou et al. 2012). *E. coli dcm* mutants display increased expression of the stress response sigma factor, RpoS, suggesting cytosine methylation may be involved in gene expression and the stress response (Kahramanoglou et al. 2012). Further, the absence of a solitary cytosine methyltransferase (5mC), HpyA-VIBM, in *Helicobacter pylori* alters the expression of genes involved in motility, adhesion, and virulence (Kumar et al. 2012).

5.2.2.3 CcrM Methylase

The role of DNA adenine methylation in cell-cycle-related events has been extensively studied in *Caulobacter crescentus*, serving as a model organism for bacterial cell-cycle regulation and development (Gonzalez et al. 2014; Marczynski and Shapiro 2002; McAdams and Shapiro 2003; Reisenauer et al. 1999). *C. crescentus* is a member of the *Alphaproteobacteria*, which includes *Brucella abortus* (brucellosis), *Agrobacterium tumefaciens* (crown gall disease), and *Sinorhizobium meliloti* (nitrogen-fixation). It has a dimorphic life cycle, spending part of its life cycle as a non-replicating motile swarmer cell and the other as a replicating sessile stalked cell. Many of the cellular events leading to differentiation into these morphological stages are modulated by the solitary cell-cycle regulated methyltransferase, CcrM, which methylates the N^6 position of adenine in the sequence GANTC (Marczynski and Shapiro 2002; McAdams and Shapiro 2003; Reisenauer et al. 1999). The *C. crescentus* chromosomal methylation state (unmethylated, hemimethylated, fully methylated) controls a regulatory cascade that couples DNA replication and the expression of cell-cycle master regulators, which facilitate progression of the *Caulobacter* cell cycle (Collier et al. 2006).

5.3 Dam Methylation Modulates the Timing and Targeting of Cellular Processes

Dam plays a role in the timing and targeting of many cellular processes including DNA repair, DNA replication, transposition, conjugation, as well as those specifically involved in bacterial virulence (Løbner-Olesen et al. 2005; Low and Casadesús 2008; Low et al. 2001; Marinus and Casadesús 2009; Sánchez-Romero et al. 2015).

5.3.1 Dam Controls DNA Repair and Replication

Errors that occur during replication are corrected by methyl-directed mismatch repair that can distinguish base mismatches on the newly synthesized strand. Such DNA strand discrimination is accomplished using hemimethylated DNA that arises after passage of the replication fork, whereby the parental strand is methylated at Dam-target sequences (GATC sites) and the newly synthesized strand is non-methylated (Pukkila et al. 1983). DNA base mismatches on newly synthesized DNA are recognized and removed by the MutHLS DNA mismatch repair proteins, and the errors are corrected using the parental strand as a template (Iyer et al. 2006). Subsequently during the cell cycle, the newly synthesized strand is methylated by Dam at GATC sites resulting in fully methylated DNA. Dam levels are controlled primarily at the transcriptional level (Løbner-Olesen et al. 2003) and the absence, or overproduction, of Dam leads to an increase in spontaneous mutation frequency due to the lack of hemimethylated DNA needed for strand discrimination during DNA mismatch repair (Heithoff et al. 2007; Herman and Modrich 1982; Marinus and Morris 1974).

The timing of DNA replication is controlled by a competition between Dam and DNA-binding proteins that recognize hemimethylated DNA. SeqA binds specifically to several hemimethylated GATC sites at and near the origin of replication (*oriC*), delaying their methylation by Dam (Kang et al. 1999; Lu et al. 1994). The sequestration of these hemimethylated sites by SeqA delays further replication fork initiation since it represses transcription of the replication initiator (*dnaA)* and inhibits DnaA binding at *oriC* as both processes operate optimally at fully methylated GATC sites (Marinus and Casadesús 2009). Additionally, SeqA acts at hemimethylated sites to play a role in nucleoid structure, organization, and partitioning into daughter cells (Bach et al. 2003; Helgesen et al. 2015; Joshi et al. 2013; Skarstad and Katayama 2013). Thus, competition between Dam and DNA-binding proteins controls the timing and targeting of many cellular events that are critical to the cell cycle.

5.3.2 Dam Controls Bacterial Gene Expression

Dam methylation of GATC sites can control gene expression via altering the affinity of DNA-binding proteins to regulatory sequences, as described in the following examples (Casadesús and Low 2006; Løbner-Olesen et al. 2005; Low and Casadesús 2008; Low et al. 2001; Marinus and Casadesús 2009; Sánchez-Romero et al. 2015).

Initiation of DNA Replication Sequestration of hemimethylated GATC sites by SeqA at the *oriC* region delays replication fork initiation via *dnaA* transcriptional repression and DnaA-binding inhibition at *oriC* (discussed above). *Implications*: Maintenance of hemimethylated DNA near the *oriC* region limits the number of replication forks that can initiate before cell division.

Transposition Tn*10* transposition occurs upon the generation of hemimethylated GATC sites in the transposase promoter (Roberts et al. 1985). The transposase promoter is only active when the transposase-coding strand is methylated and the noncoding strand is not methylated. *Implications*: Transposition is repressed through most of the cell cycle, preventing high-level transposition that would otherwise cause detrimental effects to the genome. Transposition is limited to one copy while the other copy remains in the original location.

Conjugal Plasmid Transfer Stimulation of the *tra* operon for conjugal transfer of the *Salmonella* virulence plasmid occurs upon generation of hemimethylated GATC sites within the upstream regulatory sequences for *traJ* expression, a transcriptional activator of the *tra* operon. Methylation of the noncoding strand (but not the coding strand) stimulates binding of the leucine-responsive regulatory protein (Lrp), with resultant *traJ* transcription, and conjugal transfer of the methylated noncoding single-stranded DNA into the recipient bacterium (Camacho and Casadesús 2002; Camacho and Casadesús 2005). *Implications*: Conjugal transfer is repressed through most of the cell cycle via a *traJ* epigenetic switch, thereby modulating the considerable metabolic and energetic cost of mating functions to the cell. Recipient cells are competent for conjugation since the noncoding, methylated strand serves as a template for DNA replication, reproducing the DNA methylation pattern that permits Lrp binding.

Cell Invasion *Salmonella* invasion of human epithelial cells is impaired in the absence of Dam methylation (Garcia-Del Portillo et al. 1999). Binding of the HdfR regulatory protein to unmethylated GATC sites in regulatory sequences for the *std* fimbrial operon stimulates StdEF-mediated repression of invasion determinants encoded on *Salmonella* Pathogenicity Island I (SPI-1) (Jakomin et al. 2008; López-Garrido and Casadesús 2012). *Implications*: Methylation state of invasion-associated regulatory sequences ensures bacterial invasion of only appropriate cells/cellular compartments that contribute to the onset and progression of infection.

Pili Phase Variation Dam methylation controls the production of *E. coli* pyelonephritis-associated pili (*pap*) via a phase-variation mechanism that results in cells that either express or do not express the pili. Dam is in competition with two transcriptional activators (Lrp, PapI) for GATC sites in upstream regulatory sequences for *pap* expression, forming DNA methylation patterns that can be inherited in progeny populations similar to that observed in eukaryotes (Low and Casadesús 2008; Marinus and Casadesús 2009). *Implications*: Phase variation (ON-OFF) control of pili adherence via Dam methylation results in periods of bacterial attachment and detachment, facilitating uropathogenic *E. coli* progression from the bladder to kidney, resulting in pyelonephritis.

5.4 DNA Methylation Plays an Essential Role in Bacterial Virulence

DNA methylation has been shown to play a role or has been implicated in the virulence of many bacterial pathogens (Casadesús and Low 2006; Heusipp et al. 2007; Low et al. 2001; Marinus and Casadesús 2009; Sánchez-Romero et al. 2015). Representative examples are discussed below, including pathogens that utilize solitary or R-M methyltransferases to modulate bacterial virulence.

5.4.1 DNA Methylation Controls Bacterial Pathogenesis

Salmonella spp. Nontyphoidal *Salmonella* (NTS) is the greatest foodborne-disease burden in the United States, with greater than one million illnesses annually (Gilliss et al. 2011; Scallan et al. 2011). *Salmonella enterica* infection can result in any of four disease syndromes: enterocolitis/diarrhea, bacteremia, typhoid fever, and chronic asymptomatic carriage (Coburn et al. 2007). Many serovars infect both humans and animals, with the particular syndrome a function of the serovar (serotypic variant), strain virulence, and host susceptibility (Coburn et al. 2007; Heithoff et al. 2012). Dam methylation plays an essential role in *Salmonella* virulence (Garcia-Del Portillo et al. 1999; Heithoff et al. 1999). The lack or overproduction of Dam confers significant virulence attenuation (10,000-fold) in murine models of typhoid fever. Dam methylation is involved in the invasion of nonphagocytic cells, M-cell cytotoxicity, bile resistance, envelope stability, cell motility, fimbrial, O-antigen and cytotoxin production, systemic dissemination, and the elicitation of host innate and adaptive immune responses (Badie et al. 2007; Garcia-Del Portillo et al. 1999; Heithoff et al. 1999, 2001, 2007, 2008; López-Garrido and Casadesús 2010; Pucciarelli et al. 2002; Sarnacki et al. 2009; Shtrichman et al. 2002; Simon et al. 2007). *Implications*: Dam methylation controls the production of many factors underlying microbial virulence (adhesins, invasins,

toxins) and impacts host–pathogen interactions that compromise host immunity. *Salmonella dam* mutants are capable of eliciting cross-protection against a diversity of salmonellae and are well-tolerated when applied as modified live vaccines in mice (Heithoff et al. 2001, 2008, 2015), poultry (Dueger et al. 2001, 2003a), sheep (Mohler et al. 2011) and calves (Dueger et al. 2003b; Mohler et al. 2006, 2008). Induction of immunity is rapid, and the vaccine can be delivered in drinking water for low-cost and low-stress vaccination of livestock populations (Mohler et al. 2011, 2012).

Yersinia spp. *Yersinia pseudotuberculosis* and *enterocolitica* are zoonotic foodborne pathogens that can cause severe disease in humans including gastroenteritis, mesenteric lymphadenitis, and septicemia (Galindo et al. 2011; Tauxe 2015). Many pathogenic strains infect both humans and animals whereby the particular syndrome is a function of the serotype, strain virulence, and host susceptibility. The *dam* gene is essential in certain strains of *Yersinia* species, and the lack or overproduction of Dam in *Y. pseudotuberculosis* leads to severe virulence attenuation in murine models of bacteremia (Julio et al. 2001; Kubicek-Sutherland et al. 2014; Taylor et al. 2005) and confers protection to heterologous *Y. pseudotuberculosis* or *Y. pestis* challenge (Julio et al. 2001; Kubicek-Sutherland et al. 2014; Taylor et al. 2005). Dam overproducing *Y. pseudotuberculosis* ectopically secrete several *Yersinia* outer proteins (e.g., YopE cytotoxin) as well as LcrV, a low-calcium-responsive virulence factor normally involved in Yop synthesis, localization, and suppression of host inflammatory activities (Badie et al. 2004; Julio et al. 2001, 2002). Dam overproducing *Y. enterocolitica* confer altered invasion, motility, and composition of the lipopolysaccharide (LPS) O-antigen and also display ectopic Yop secretion via increased ClpP protease degradation of the LcrG regulatory protein that normally blocks Yop secretion (Fälker et al. 2005, 2006, 2007). *Implications*: Dam methylation controls the strict environmental regulation of *Yersinia* virulence function synthesis and localization, serving to modulate bacterial pathogenesis and host inflammatory activities.

Enterohemorrhagic E. coli EHEC are a subgroup of Shiga toxin-producing *E. coli* (STEC) that can cause severe intestinal disease [*i.e*., hemorrhagic colitis [HC] and hemolytic uremic syndrome [HUS] (Hartland and Leong 2013; Mahan et al. 2013)]. EHEC intestinal adherence requires the delivery of the type III secretion system (TTSS) effector proteins Tir and $EspF_U$ into the host cell and expression of the bacterial outer membrane adhesin, intimin. Increased adherence exhibited by *dam* mutant EHEC was correlated with increased protein levels of Tir, $EspF_U$, and intimin (Campellone et al. 2007). Dam also controls the maintenance of lysogeny for a bacteriophage (933W) that encodes Shiga toxin (Stx-2), which inhibits protein synthesis (via ribosomal inactivation) and leads to renal toxicity in HUS patients (Murphy et al. 2008). *Implications*: Dam methylation modulates EHEC intestinal adherence and Shiga toxin production during infection.

Brucella abortus *B. abortus* is an intracellular pathogen and the causative agent of brucellosis, a zoonotic disease that causes abortions and stillbirths in livestock and

acute febrile illness in humans, which may progress to chronically debilitating disease (World Health Organization 2006). It is also designated as a select agent with the potential for bioterrorism due to the chronic nature of disease in livestock and humans and its ability to undergo aerosolization (Centers for Disease Control and Prevention 2015). *B. abortus* is a member of the *Alphaproteobacteria* which have defined morphological stages that are modulated by the solitary cell cycle-regulated DNA methyltransferase, CcrM (Marczynski and Shapiro 2002). CcrM is essential for viability in *B. abortus*, and its overexpression attenuates replication within murine macrophages (Robertson et al. 2000). *Implications*: CcrM methylation may play a role in intracellular replication of the bacterium within phagocytes, a key virulence characteristic for both acute and chronic cases of brucellosis.

Mycobacterium tuberculosis *M. tuberculosis* infections cause nine million active cases and 1.5 million tuberculosis deaths annually, with one-third of the world's population having latent infections (World Health Organization 2014a). Although there are no predicted *dam* homologues, the *M. tuberculosis* solitary DNA methyltransferase, MamA, plays a role in gene expression and fitness during hypoxia, and different methyltransferases are observed in different lineages of *M. tuberculosis* (Shell et al. 2013). *Implications*: DNA methylation may play a role in *M. tuberculosis* lineage-specific differences in preferences for distinct host environments and different disease courses in humans.

Haemophilus influenzae Non-typeable *H. influenzae* (NTHi) is a major cause of middle ear (otitis media) infections in children (Haggard 2008). NTHi contains R-M systems comprised of a methyltransferase (*mod*) and a restriction endonuclease (*res*) (Srikhanta et al. 2010). Phase variable (ON-OFF) switching of *mod* alleles (due to the presence of tandem repeats in the corresponding *mod* genes) regulates the expression of multiple proteins that are involved in antibiotic resistance, biofilm formation, and immune evasion. Recent studies indicate that *mod* switching to the ON orientation was highly selected in a chinchilla model of otitis media, and ON phase-variants formed more robust biofilms in vitro (Atack et al. 2015). These findings suggest that *mod* is involved in bacterial virulence, immune evasion, and niche adaptation. Several other human pathogens contain phase-variable R-M systems, including *H. pylori* (atrophic gastritis), *Neisseria meningitidis* (meningitis), *N. gonorrhoeae* (gonorrhea), and *Moraxella catarrhalis* (otitis media) (Atack et al. 2015; Srikhanta et al. 2010). *Implications*: Phase-variable R-M systems that modulate microbial virulence traits may be shared across the microbial realm.

5.5 Perspectives: Epigenetic Programming of the Pathogen and Disease Susceptibility

Bacterial pathogenesis can be regarded as a developmental program (Casadesús and D'Ari 2002; Mahan et al. 2010) similar to eukaryotic cell differentiation and development (Bird 2002, 2007; Jaenisch and Bird 2003). Bacterial epigenetic programming is imparted by DNA methylation, whereby the virulence traits expressed are dependent on the aggregate of interactions between the microbe and its environment. Thus, the bacterial epigenome provides a means for bacterial "memory," engendering the capacity for adaption to the disparate microenvironments encountered as the infection proceeds due to dissemination to new host sites, tissue breakdown, inflammation, and immune clearance mechanisms. Thus, a microbial population may comprise a spectrum of genotypically identical cells with significant phenotypic differences in virulence traits since pathogenicity may be a reflection of cumulative exposure to selective pressures within host(s) and environments experienced during the microbial life cycle. Epigenetic programming may provide insights into the virulence disparities of closely related strains that exhibit marked differences with regard to pathogenicity, host range, and preferences for distinct host environments and different disease courses in humans.

5.6 Microbial Infection, Epigenetic Reprogramming, and Human Disease

Microbe-associated changes in the host epigenome can play a significant role in human disease via chromatin remodeling and resultant transcriptional reprogramming driven by host DNA methylation, histone modifications, chromatin-associated complexes, and noncoding RNA-mediated silencing (Bannister and Kouzarides 2011; Bierne et al. 2012; Dawson and Kouzarides 2012; Herceg et al. 2013; Paschos and Allday 2010). DNA methylation occurs at the 5′ position of cytosines within CpG dinucleotides, and can recruit protein complexes that can alter chromatin structure or affect the binding of transcription factors with resultant gene silencing (Bird 2002, 2007; Dawson and Kouzarides 2012; Jones and Takai 2001). Histone modifications (e.g., methylation, acetylation, phosphorylation, ubiquitination) can alter chromatin structure and affect gene expression (Bannister and Kouzarides 2011; Dawson and Kouzarides 2012). Noncoding RNA-mediated silencing involves microRNAs (noncoding, 18–25 nucleotides) that target mRNAs and negatively control gene expression (He and Hannon 2004; Sato et al. 2011).

Such transcriptional reprogramming can alter host defense genes involved in TLR (Toll-like receptor), MAPK (mitogen-activated protein kinase), interferon (IFN), and NF-kB signaling pathways (Gómez-Díaz et al. 2012; Paschos and Allday 2010; Stein 2011). For instance, *M. tuberculosis* inhibits IFN-γ-induced

chromatin remodeling by TLR2 and MAPK signaling (via inhibition of histone acetylation), leading to reduced expression of several immune genes and resultant persistence of chronic infections (Pennini et al. 2006). Influenza virus suppresses the antiviral response via the production of a histone mimic that serves as a sink for a host transcription factor (hPAF1) involved in antiviral gene expression (Marazzi et al. 2012). Conversely, some microbe-associated epigenome changes are protective. Following acute viral infection, chromosome remodeling is implicated in the formation of memory CD8+ T cells that provide the host with long-term protective immunity against the pathogen (Youngblood et al. 2010).

5.6.1 Microbial Infection and Cancer

Microbe-associated cancers account for a significant proportion (>20%) of all human cancers (Moore and Chang 2010; zur Hausen 2009). The molecular basis involves microbe-stimulated changes in the host epigenome, with resultant changes in host chromatin remodeling, gene expression, and metabolism (Dawson and Kouzarides 2012; Esteller 2008; Feinberg and Tycko 2004; Herceg et al. 2013; Stein 2011).

Hepatitis B Virus (HBV) Liver cancer is the second leading cause of cancer death worldwide (World Health Organization 2014b, 2015). The risk for liver cancer is increased 100-fold in individuals with chronic HBV infection (Fernandez et al. 2009), and recent estimates indicate that ~250 million individuals in the human population are chronically infected with HBV (Schweitzer et al. 2015). HBV persists in host cells by the nuclear accumulation of covalently closed circular DNA (cccDNAs) that serve as a template for transcription of all viral mRNAs and are organized into minichromosomes by histones and nonhistone viral and cellular proteins (Grimm et al. 2011; Protzer 2015). High viral loads in patients with chronic hepatitis correlate with hyperacetylation of histone H3 and H4 bound to cccDNA in liver biopsy samples (Pollicino et al. 2006), allowing access of the HBV cccDNA chromatin-like structure to liver-specific transcription factors and subsequent replication (Quasdorff et al. 2008). The HBx regulatory protein (Kew 2011) relieves chromatin-mediated transcriptional repression of HBV cccDNA that involves the histone methyltransferase, SETDB1 (Rivière et al. 2015). HBx also upregulates several DNA methyltransferases (DNMTs), resulting in increased promoter methylation and repression of tumor-suppressor genes encoding p16, a cyclin-dependent kinase inhibitor that functions in cell-cycle arrest and cellular senescence, and E-cadherin, a cell–cell adhesion molecule that affects tumor invasiveness (Fernandez and Esteller 2010; Jung et al. 2007; Tian et al. 2013). Patient samples from various stages of HBV infection show increased methylation of the HBV genome as an acute infection transitions to a chronic infection and during the subsequent progression to premalignant lesions and cancer (Fernandez and Esteller 2010; Fernandez et al. 2009; Stein 2011). Additionally, microRNA (miR-152),

whose normal function is to downregulate DNMT1, is downregulated in patients with HBV-associated liver cancer, thus causing DNA hypermethylation (Huang et al. 2010; Saito et al. 2014). These findings suggest a tumor-suppressive role of miR-152, and therapeutic use of this microRNA may reduce aberrant DNA methylation.

Human Papillomavirus (HPV) Genital human papillomavirus (HPV) is the most commonly diagnosed sexually transmitted infection in the United States and is associated with 95% of cervical and anal cancers and 60% of oropharyngeal cancers. (Centers for Disease Control and Prevention 2012; Gilmer 2015). Through preexisting lesions, HPV infects the basal (lower) layer of the stratified cervical epithelium, and viral genomes are maintained as episomal DNA in the nuclei of infected cells (Kajitani et al. 2012). HPV oncoproteins E6 and E7 inactivate p53 and retinoblastoma (pRb) tumor-suppressing proteins, respectively, resulting in aberrant proliferation and delayed differentiation of infected host cells (Münger et al. 2004). The productive phase of the lifecycle (genome amplification, virion assembly/release) occurs in upper layers of the cervical epithelium that are terminally differentiated. In infections with HPV "high-risk" invasive serotypes (16 and 18), progression of the disease is associated with increased methylation of the HPV genome and considerable suppression of E-cadherin (Anayannis et al. 2015; Fernandez and Esteller 2010; Fernandez et al. 2009; Sun et al. 2011; Wilson et al. 2013). E-cadherin is utilized by Langerhans cells (antigen processing/presentation) to move through stratified epithelium, and its reduction may impact HPV clearance and the length of persistent infections. In a keratinocyte cell line, the HPV E7 oncoprotein is necessary for E-cadherin downregulation via augmentation of host DNMT1 levels and resultant E-cadherin repression (Laurson et al. 2010). DNMT inhibition (via 5-aza-deoxycytidine administration) restored E-cadherin levels, suggesting that epigenetic intervention may have utility in combating persistent infections via restoring influx of Langerhans cells to infected tissue. Further, epigenetic alterations to the viral genome via methylation of viral promoter regions have been implicated in HPV E6 and E7 expression during a transforming infection (Steenbergen et al. 2014). The overall consequence of deregulated expression of E6 and E7 in proliferating cells is chromosomal instability, leading to accumulation of lesions in host cell cancer genes and subsequent progression toward cancer (Korzeniewski et al. 2011).

Epstein–Barr Virus (EBV) EBV is carried in the vast majority (>90%) of the human population as an asymptomatic lifelong infection, yet it is also correlated with several nonmalignant and malignant diseases (Odumade et al. 2011; Rickinson et al. 2014; Thompson and Kurzrock 2004; Thorley-Lawson 2015). EBV causes mononucleosis and many human tumors of B cell, T cell, and epithelial origin such as Burkett's lymphoma, Hodgkin's disease, gastric carcinoma, nasopharyngeal carcinoma, and lymphoproliferative tumors in immunocompromised individuals. The EBV lifecycle involves infection of oropharyngeal cells; host colonization through growth-transforming latent infection of B cells within oropharyngeal lymphoid tissues; long-term persistence within recirculating memory B cells as a

silent latent infection; and reactivation to the viral lytic phase and subsequent infection of naïve host cells (Rickinson et al. 2014). Many of these events are driven by epigenetic reprogramming of the pathogen and host, whereby B cell growth transformation is facilitated by several latent proteins, including EBV nuclear antigens (EBNAs) and latent membrane proteins (LMPs), followed by regulated shutdown of latent protein expression that ultimately results in latency in recirculating B cells (Hammerschmidt 2015; Paschos and Allday 2010). Increased methylation of the EBV genome occurs as an acute infection transitions to chronic infection and during subsequent development and progression of cancer (Fernandez and Esteller 2010; Fernandez et al. 2009), and infection of B lymphocytes or nasopharyngeal carcinoma cell lines results in the expression of several DNMTs (Schmeinck 2011; Tsai et al. 2006). During the latent phase, EBV lytic genes are transcriptionally silenced by histone methyltransferase EZH2, a component of the Polycomb Repressive Complex 2, PRC2; and these "histone marks" are erased upon lytic phase induction (Hammerschmidt 2015; Woellmer et al. 2012). These findings indicate that epigenetic modifications of viral DNA determine viral latency.

Helicobacter pylori Stomach cancer is the third leading cause of cancer death worldwide (World Health Organization 2014b, 2015). *H. pylori* is a gastric pathogen that colonizes approximately 50% of the world's population (Wroblewski et al. 2010), associated with 65% of gastric cancers, and classified as a class I carcinogen (Polk and Peek 2010; World Health Organization 2014b). In patients infected with *H. pylori*, aberrant methylation and repression of tumor-suppressor genes (E-cadherin, p16) was linked with increased gastric cancer risk (Kaise et al. 2008; Maekita et al. 2006; Nakajima et al. 2006; Yoshida et al. 2013). In a gerbil model of gastric cancer, *H. pylori* infection was shown to be causally involved in the induction of aberrant methylation in the host epigenome, which was associated with the upregulation of several inflammation-related genes (*CXCL2, IL-1β, NOS2, TNF-α*) (Niwa et al. 2010). Methylation decreased upon bacterial clearance but remained significantly higher than that observed in uninfected control animals. Suppressing inflammation with the immunosuppressive drug, cyclosporin A, prevented aberrant methylation without affecting colonization, indicating that epigenetic modifications occurred as a consequence of inflammation rather than the infection itself. These studies revealed an "epigenetic field defect" whereby increased DNA methylation that arises as a result of infection marks a region with higher risk for transformation (Niwa et al. 2010; Stein 2011). Thus, DNA methylation has potential clinical utility as a biomarker for the risk of malignant transformation for a number of cancers, offering new therapeutic opportunities that target and monitor epigenetic changes (discussed below).

5.7 Concluding Remarks: Microbial Infection and Its Impact on the Host Epigenome and Disease

The origin of some diseases may have a microbial component even though there may be no apparent direct link between infection and disease. How does this occur and what are the possible implications? Microbial infection can trigger heritable changes in the host epigenome that lead to profound differences in disease susceptibility, host cell metabolism, inflammation, and immune responses, and some of these responses may be maintained long after microbial clearance (Bierne et al. 2012; Davis et al. 2011; Stein 2011). The primary challenge toward establishing a causal link between infection-associated changes in the host epigenome and disease origin is the considerable interplay between epigenetic, genetic (mutational), and nonmicrobial (*e.g.*, carcinogen) risk factors that cloud the assignment of primary versus secondary events leading to disease development and progression (Fig. 5.1). Microbes cause cancer directly via harboring oncogenes that contribute to cell transformation or indirectly through chronic inflammation whereby ultimately carcinogenic mutations are generated in host cells (Moore and Chang 2010; Parsonnet 1999; zur Hausen 2001). Additionally, microbial and nonmicrobial associated alterations in host epigenetic determinants influence many biological

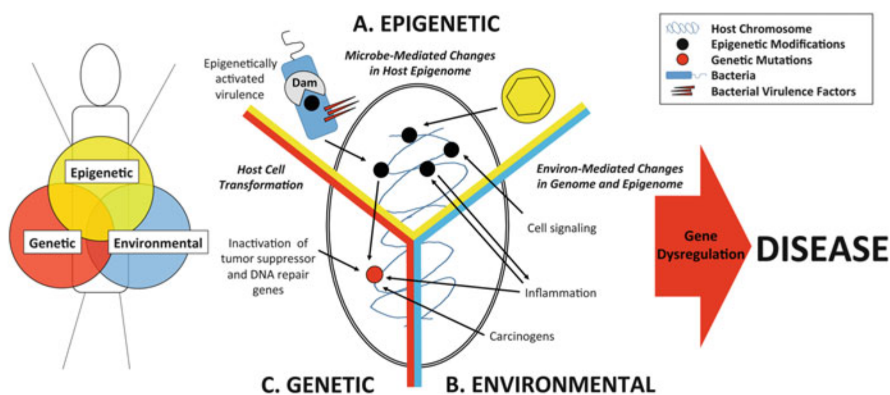

Fig. 5.1 Epigenetic programming of the pathogen and the host can stimulate the development and progression of acute and chronic disease. (**a**) The epigenome of pathogenic microbes can be modified to stimulate the production of virulence determinants (via Dam; host DNMTs). Pathogenic bacteria can modify the host epigenome (*dark circles*) via DNA methylation, histone modifications, chromatin-associated complexes, and noncoding RNA mediated silencing. (**b**) Environmental inputs can alter disease susceptibility by stimulating genetic (mutational; *red circles*) or epigenetic changes (nonmutational; *dark circles*) in the host genome via carcinogen exposure, cell signaling, and inflammation. (**c**) Chronic disease (*e.g.*, cancer) can be stimulated directly by genetic changes in the host genome caused by exposure to carcinogens, microbial oncogenes, and chronic inflammation or indirectly via epigenetic changes in the host genome by inactivation of tumor-suppressor genes and/or DNA-repair genes, which predispose the genome to mutation. Such complex interactions between genetic, epigenetic, and environmental inputs result in host gene dysregulation and human disease

processes that are fundamental to the development of cancer including the inactivation of tumor-suppressor genes and/or DNA-repair genes, which predispose the genome to mutation (Baylin and Herman 2000; Dawson and Kouzarides 2012; Herceg et al. 2013; Moore and Chang 2010; Paschos and Allday 2010; Romani et al. 2015; Stein 2011).

Despite these challenges, significant advances have been made toward establishing a direct link between microbe-associated changes in the host epigenome and cancer, and it remains a possibility that certain disorders are a consequence of chronic inflammation with microbial origin (Bierne et al. 2012; Costenbader et al. 2012; Elinav et al. 2013; Feinberg and Tycko 2004; Grivennikov et al. 2010; Herceg et al. 2013; Liu et al. 2008; Portela and Esteller 2010; Schett et al. 2013; Ushijima and Hattori 2012; Van Vliet et al. 2007; Wilson 2008). For example, the gut microbiome (the largest reservoir of microbes in the body) stimulates host epigenome changes that are linked to inflammatory bowel disease (Khor et al. 2011; Knights et al. 2013; Kostic et al. 2014; Ventham et al. 2013). Since there are ~100 trillion microbial cells in the gastrointestinal tract—roughly ten times more than the cells in the human body—the gut microbiome has the capacity to produce a variety of compounds that can impact host genomic/epigenomic processes and metabolism (Bianconi et al. 2013; Garagnani et al. 2013; Shenderov 2012; Stilling et al. 2014). Examples include microbial structural components and metabolites (*e.g.*, peptides, polysaccharides, endotoxins, short-chain fatty acids, co-factors) that are potential epigenomic modifiers, which can affect gene expression and metabolism in the host via transcriptional reprogramming of host signaling pathways (Gómez-Díaz et al. 2012; Knights et al. 2013; Paschos and Allday 2010; Stein 2011). Notably, host–gut microbe interactions can lead to considerable systemic signaling, involving many organs and organ systems, including the central nervous system (Stilling et al. 2014). Thus, the role of epigenetics in host–microbe interactions leading to pathological syndromes—with the potential of the disruption of homeostasis due to pathogen exposure—provides the foundation for the development of new medicines and diagnostic tests for diseases with epigenomic determinants.

The significant challenge of epigenetic therapies lies in the lack of specificity—and the global hypomethylation achieved by DNMT inhibitors—which may be detrimental to developing an effective treatment. Notwithstanding, cancer treatment applications include administration of small molecules that inhibit epigenetic factors (Dawson and Kouzarides 2012; Romani et al. 2015), risk assessments that link the degree of aberrant DNA methylation to the likelihood of cell transformation (Niwa et al. 2010; Stein 2011), and gene therapy targeting epigenetic factors (Yao et al. 2015). The use of "epigenetic modifier drugs" may extend beyond cancer to other epigenetically based diseases as evidenced by their current testing in noncancer clinical trials (*e.g.*, irritable bowel syndrome, Alzheimer disease, cardiovascular disease, thalassemia, psoriasis) (Romani et al. 2015). Additionally, combinational therapies—comprising epigenetic modifier drugs and antimicrobials—may prove useful in combating infectious diseases and associated disease manifestations such as blood clotting and inflammation that can cause severe tissue

damage and organ failure leading to death (Grewal et al. 2013; Herceg et al. 2013; Moore and Chang 2010; Schleithoff et al. 2012; Yang et al. 2015).

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