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Host-microbiota interactions: Epigenomic regulation

Vivienne Woo and Theresa Alenghat¹

¹Division of Immunobiology, Cincinnati Children's Hospital Medical Center and the University of Cincinnati College of Medicine, Cincinnati, OH 45229 USA

Abstract

The coevolution of mammalian hosts and their commensal microbiota has led to the development of complex symbiotic relationships between resident microbes and mammalian cells. Epigenomic modifications enable host cells to alter gene expression without modifying the genetic code, and therefore represent potent mechanisms by which mammalian cells can transcriptionally respond, transiently or stably, to environmental cues. Advances in genome-wide approaches are accelerating our appreciation of microbial influences on host physiology, and increasing evidence highlights that epigenomics represent a level of regulation by which the host integrates and responds to microbial signals. In particular, bacterial-derived short chain fatty acids have emerged as one clear link between how the microbiota intersects with host epigenomic pathways. Here we review recent findings describing crosstalk between the microbiota and epigenomic pathways in multiple mammalian cell populations. Further, we discuss interesting links that suggest that the scope of our understanding of epigenomic regulation in the host-microbiota relationship is still in its infancy.

Introduction

Environmental factors have been implicated in driving development and pathogenesis of many chronic human diseases with complex multifactorial etiologies, such as asthma, allergy, inflammatory bowel disease, diabetes and cancer [1–3]. Alterations in the diversity of the microbiota (dysbiosis) have been widely associated with many of these chronic human conditions, highlighting that the microbiota and microbiota-derived signals act as environmental cues that influence host physiology. Trillions of commensal microbes reside in the intestinal tract and are critical in modulating local and systemic immune responses [4,5]. Close association between the microbiota and the single layer of intestinal epithelial cells (IECs) that line the intestine are also necessary to regulate essential biological processes such as metabolism, nutrient uptake, neuronal development and angiogenesis [6–8]. Epigenomic modifications are central mechanisms involved in directing transcriptional response to environmental cues, and thus represent a potentially significant interface by which the microbiota can dynamically interact with the host genome. Further, understanding

CORRESPONDENCE: Theresa Alenghat, theresa.alenghat@cchmc.org; 513-803-7498.

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how underlying epigenomic pathways are regulated by the intestinal microbiota could aid in identifying potential therapeutic targets to prevent and treat health conditions associated with an altered host-microbiota relationship. Here, we review recent advances in our understanding of host-microbiota interactions, focusing on epigenomics as a key mechanism guiding microbe-dependent mammalian physiology.

Basics of Epigenomics

Epigenomics is the study of molecular mechanisms that dynamically and reversibly alter a cell's transcriptional potential without altering the underlying genetic sequence. Epigenomic regulation is often associated with development and facilitating tissue/cellular plasticity [9–11], but recently, changes in a mammalian cell's epigenome have been highlighted in the context of transcriptional control by external environmental signals. Within the nucleus of eukaryotic cells, DNA is condensed into a higher order structure termed chromatin. Nucleosomes, the basic repeating units within chromatin, contain DNA wound around a histone octamer (H2A, H2B, H3, and H4), followed by a linker histone H1 that joins adjacent nucleosomes together. Changes in chromatin state through condensation and relaxation allow for DNA replication and repair [12,13]. In addition, gene accessibility based on chromatin conformation and modifications has a substantial influence on a cell's transcriptional program. In general, condensed chromatin (heterochromatin) limits the recruitment of the transcriptional machinery to the DNA and results in decreased expression of associated genes, while open chromatin (euchromatin) is more commonly enriched with actively transcribed genes [14].

Structural reorganization of the chromatin is mediated by ATP-dependent remodeling enzymes and covalent epigenomic modifications in response to endogenous and environmentally-derived signals [15]. The most well characterized examples of covalent epigenomic modifications are DNA methylation and histone modifications such as acetylation, methylation, phosphorylation, SUMOylation and ubiquitination. These modifications are put in place by DNA or histone modifying enzymes such as DNA methyltransferases (DNMTs) and histone methyltransferases, and are maintained by the balanced activity of opposing enzymes (i.e. histone acetyltransferases versus histone deacetylases (HDACs)). HDACs have recently been examined as targets of microbiota-derived metabolites and therefore these epigenome-modifying enzymes are discussed in more detail below. Collectively referred to as the “histone code”, the pattern of epigenomic modifications can direct chromatin restructuring and transcription factor recruitment and, thus, epigenomics is thought to represent a central mechanism by which the environment impacts mammalian gene expression in health and disease [16,17].

Microbiota-derived metabolites: Short-chain fatty acids (SCFAs)

Mammalian cells can sense microbes through pattern recognition receptors such as toll-like receptors (TLRs) that recognize lipopolysaccharide (LPS). Recently, the microbial-derived short chain fatty acids (SCFAs) that are produced by commensal bacteria, such as *Clostridia* and *Bifidobacteria*, from fermentation of carbohydrates and fiber have emerged as central players mediating crosstalk between the microbiota and host [18,19]. In particular,

propionate, acetate and butyrate, the three most abundant SCFAs in the intestinal lumen, have received increasing attention in the field due to their potential beneficial impact on host physiology including reduced inflammation and enhanced epithelial barrier function, although these effects have varied between studies [3,20–24]. Germ-free mice express little to no SCFAs, indicating that production of these metabolites is dependent on the microbiota [25]. Although their mechanism of action is not fully understood, SCFAs are thought to modulate host cellular processes through (1) direct inhibition of HDAC activity and/or (2) activation of G-protein-coupled-receptors (GPCRs) [25–27]. HDACs remove acetyl residues from histone or non-histone proteins and the eighteen known mammalian HDACs are classified into four classes based on sequence homology. These epigenomic-modifying enzymes are often present in large protein complexes that are guided to target chromatin through transcription factor interactions. Given that SCFAs, and particularly butyrate, have long been known to broadly inhibit the HDAC epigenomic family of enzymes, several recent studies have demonstrated or suggested that SCFAs mediate host-microbiota interactions through epigenomic regulation. Therefore, studies related to SCFAs will be discussed in more detail below.

Epigenomic mechanisms regulate microbiota-dependent immune homeostasis

Interactions between the host and microbiota through epigenomic regulation are best characterized in the hematopoietic immune system and reviewed extensively elsewhere [28,29] (Fig. 1). Macrophages and dendritic cells (DCs) are critical in innate barrier defense against invading pathogens, and rapidly alter their transcriptional profile in response to bacterial colonization. Histone H3 lysine 4 trimethylation (H3K4me3) is an epigenetic mark associated with enhanced gene expression, and non-mucosal mononuclear phagocytes isolated from conventionally-housed mice displayed increased H3K4me3 levels at the transcriptional start site of pro-inflammatory genes such as interleukin 6 (*IL-6*) and interferon beta 1 (*Ifnb1*). These epigenomic changes corresponded with increased expression of *IL-6* and *Ifnb1* and enhanced priming of natural killer cells in conventionally-housed mice relative to germ-free mice [30]. Microbiota-induced increase in colonic HDAC3 expression and increased histone deacetylation on the *Il12b* promoter in intestinal macrophages were found to be important in the IL-10-mediated inhibition of IL-12 expression and intestinal inflammation [31]. Global histone acetylation was also shown to be increased in intestinal macrophages in response to microbial-derived butyrate, corresponding with decreased expression of *IL-6* and *IL-12* [32]. These genes, among others, were also downregulated in macrophages from antibiotic-treated mice, and exhibited impaired interferon signaling and anti-viral function compared to conventionally-housed mice [33]. Recent work also demonstrated a novel role for butyrate in facilitating colitis-protective M2 macrophage polarization *in vivo*, as well as reducing TNF- α and IL-1 β production to suppress inflammation [34]. Moreover, *in vitro* experiments on bone marrow-derived macrophages indicated that M0 to M2 differentiation was induced by butyrate, potentially through inhibition of HDACs and enhanced histone H3K9 acetylation and IL-4/STAT4 signaling [34]. Importantly, decreased gene expression in response to butyrate is not consistent with the generally expected outcome of HDAC inhibition and increased histone acetylation,

indicating that butyrate is possibly functioning through other mechanisms in addition to HDACs or that butyrate-induced increased histone acetylation at specific genes mediates decreased gene expression. Nonetheless, these studies collectively support that transcription of critical macrophage-derived factors is induced through microbiota-triggered histone modifications.

DCs are also epigenetically responsive to microbial signals, displaying increased global H3 acetylation and reduced expression of *IL-6*, *IL-12* and transcription factor *Relb* following exposure to butyrate [35] (Fig. 1). This pathway was also found to mediate regulatory T cell (Treg) differentiation, discussed in more detail below. Butyrate activation of GPR109A and CpG oligodeoxynucleotide/TLR9 stimulation were found to modulate retinoic acid signaling in DCs [36]. Activation of retinoic acid signaling in DCs was later shown to increase enrichment of active enhancer histone marks, H3K4me1 and H3K27Ac, at the *avb8* integrin (*Itgb8*) locus in DCs, resulting in increased *Itgb8* expression and enhanced Treg generation [37].

Recent studies demonstrated that temporary exposure to segmented filamentous bacteria (SFB), a gram-positive murine commensal bacteria, induced expression of the Jumanji family-related lysine demethylase 6B (*Jmjd3/Kdm6b*) and increased H3K27me3 in bone marrow derived cells [38]. This induction was associated with persistent expansion of granulocyte/monocyte precursors. Further, administration of SFB-induced serum amyloid A (SAA) resulted in increased global H3K27me3 levels and *Jmjd3* expression and conferred protection against *Entamoeba histolytica* infection. Reciprocally, chemical inhibition of *Jmjd3* demethylase activity prevented SAA-induced global H3K27 methylation, suggesting that the epigenomic-modifying activity of *Jmjd3* may be regulated by distinct commensal bacterial populations such as SFB. Taken together, microbiota-triggered epigenomic regulation may represent a basic mechanism by which innate precursor cells maintain basal levels of immune regulatory gene expression that protects mucosal barrier function.

Innate lymphoid cells (ILCs) are a unique effector cell subset originally identified in mucosal-associated lymphoid tissues [39]. ILCs have been linked to regulation of immune and metabolic homeostasis, tissue repair, and host defense, but may also drive a pathogenic pro-inflammatory state [40–46]. There are currently three ILC subtypes (ILC1, ILC2 and ILC3) that are defined based on transcription factor and cytokine profiles [43,47]. In the intestine, ILC3s are particularly abundant and are important for epithelial antimicrobial peptide production and maintaining intestinal barrier function (Fig. 1) [39,45,48,49]. While some studies have demonstrated that ILC3s with enhanced *RORγt* and IL-22 expression can be elicited in response to commensal bacteria [40–42], others have found that normal numbers of IL-22-producing *RORγt*⁺ ILC3s are present in germ-free mice that lack a microbiota [50–52]. A series of recent studies aimed to improve our classification and understanding of ILC-subtypes by epigenetically and transcriptionally profiling ILCs isolated from both humans and mice using genome-wide tools. *Koues et al.* identified distinct lineage-defining gene regulatory pathways in ILC subsets and T helper (Th) cells isolated from human tonsils [53]. These pathways consisted of overlapping yet distinct expression signatures and corresponding epigenomic landscapes based on active promoter (H3K4me3) and enhancer (H3K27Ac) chromatin marks. Simultaneously, *Shih et al.*

demonstrated that both ILC and CD4⁺ Th cells possess unique subtype-defining chromatin landscapes that contain regulatory elements in signature genes that are consistent with lineage-specific gene expression [54]. *Gury-BenAri et al.* further elucidated ILC-heterogeneity by using unbiased single-cell transcriptome and genome-wide epigenomic analyses [55]. These analyses revealed that intestinal ILC1 and ILC2 cells isolated from germ-free or antibiotic-treated mice had increased enrichment of activating H3K4me2 marks at ILC3 lineage-defining enhancers compared to microbiota-replete mice [55]. The lack of microbiota-derived signals favored epigenomic and transcription profiles more consistent with an ILC3 phenotype, highlighting a potentially novel role for the microbiota in regulating ILCs in the intestine through epigenomics (Fig. 1).

Similar to ILCs, invariant natural killer T cells (iNKTs) respond to signals from the microbiota, produce effector cytokines, and activate adaptive immune cells [56]. Colonic lamina propria and lungs of germ-free mice were found to contain greater numbers of iNKT cells, leading to increased susceptibility to intestinal and pulmonary disease compared to microbiota-replete mice [57]. Microbiota colonization in neonatal mice resulted in decreased DNA methylation of the *Cxcl16* gene, reduced expression of *Cxcl16*, less iNKT accumulation, and improved barrier function (Fig. 1). These data were among the first to define a crucial role for neonatal exposure to the microbiota in driving epigenomic modifications with potential long-term consequences on mucosal homeostasis. Given that the most critical period for microbiota colonization and establishment occurs during the first few years of life, this data provokes the idea that early exposure to the microbiota may mediate essential epigenomic regulation in immune cells that could prevent or predispose to disease later in life.

Microbiota-derived signals epigenetically regulate T cell differentiation

Peripheral Tregs are generated from naïve CD4⁺ cells programmed to express the lineage-defining transcription factor forkhead box P3 (*Foxp3*) [58]. Interestingly, intestinal colonization by butyrate-producing *Clostridium* species, or oral administration of butyrate, was found to directly promote histone H3 acetylation of the *FoxP3* gene promoter and intronic enhancers conserved non-coding sequence (CNS) 1 and CNS3 in peripheral CD4⁺ T cells (Fig. 1) [59]. Later work demonstrated that butyrate stimulation of isolated naïve peripheral CD4⁺ T cells induced enrichment of activating histone acetylation marks (H3K27Ac) in these regulatory regions, and resulted in increased *Foxp3* expression and an increase in circulating Tregs [35]. Furthermore, acetylation of the Foxp3 protein was also increased in response to butyrate, which has been proposed to increase stability of the transcription factor [35]. Treg expansion reduced local inflammation and enhanced protection against T cell-induced colitis, suggesting a crucial role for butyrate in mediating an anti-inflammatory environment. In addition to regulating Treg differentiation and histone acetylation, SCFAs can induce effector T cell differentiation in secondary lymphoid organs by inhibiting endogenous HDAC activity independent of GPCR-activation [60]. Inhibition of HDAC activity was associated with increased IL-10, IFN- γ and IL-17 expression, thereby promoting T cell lineage-commitment to effector Th1 and Th17 T cells and Foxp3⁻ IL-10⁺ regulatory T cells. Taken together, these studies emphasize the importance of SCFAs in the regulation of T cell homeostasis.

While the role of SCFAs has been investigated, fewer studies have characterized the host epigenomic modifying enzymes that are sensitive to microbiota-derived signals. *Smith et al.* found that colonic Tregs exhibited decreased HDAC6 and HDAC9 protein and increased global histone H3K9 acetylation in response to GPR43 activation by propionate [61]. Consistently, HDAC6^{-/-}, and to lesser extent HDAC9^{-/-} Tregs demonstrated increased protein acetylation and enhanced Foxp3 stability, and associated improvement in Treg function *in vitro* and *in vivo* [62]. Treg-specific loss of another HDAC, HDAC3, decreased post-translational modification of Foxp3 protein and its stability [63]. However, the requirement for specific HDAC isoforms in Tregs is yet to be studied comparing microbiota-depleted and microbiota-replete conditions.

Besides butyrate-mediated changes in histone and protein acetylation, microbe-directed DNA methylation is also involved in Treg-cell homeostasis [64]. Specifically, expression of the DNA methylation adaptor protein UHRF1, in complex with Dnmt1 and HDAC1, was found to be increased in colonic Tregs in response to microbial colonization. T-cell specific deletion of Uhrf1 suppressed Treg development, proliferation and function, rendering the mice more susceptible to developing spontaneous colitis [64]. This finding demonstrated that microbiota-driven Uhrf1 expression regulates DNA methylation status and expression of genes involved in colonic Treg-cell homeostasis. The mechanisms by which the microbiota influence Uhrf1, HDACs, and other epigenomic modifiers in T cells are only starting to be uncovered and represent an exciting new area of research.

Epigenomic pathways directed by the microbiota maintain intestinal epithelial barrier function

In addition to immune cells, increasing data is emerging that support epigenomics as a potent mechanism by which the microbiota impacts intestinal epithelial cells (IECs). IECs line the intestinal lumen and are directly exposed to the microbiota, secrete antimicrobial peptides, and produce cytokines that regulate immune cell recruitment and function [65,66]. Studies comparing germ-free mice to microbiota-replete mice demonstrated that the microbiota and even single commensal bacterial species significantly influence gene expression in IECs (Fig. 2) [67–70]. Further, microbiota-dependent transcription in IECs varied depending on the location in the intestine, and position within the crypt-villus architecture [71]. IECs are equipped to sense microbial components through TLRs, and initial studies revealed that TLR4 expression on IECs was epigenetically regulated by the microbiota [72]. Specifically, 5' CpG methylation in the Toll-Like receptor 4 (*Tlr4*) gene was reduced in colonic IECs of germ-free mice compared to their conventionally housed counterparts, consistent with reduced Tlr4 expression and LPS hyposensitivity [72]. These data were among the first to implicate manipulation of the host epigenome by the microbiota. More recent broad assessment of IEC chromatin landscape revealed minimal difference in chromatin accessibility between germ-free mice and newly conventionalized mice [73], suggesting that microbiota-dependent epigenomic modifications may not globally impact chromatin accessibility but instead regulate transcription factor expression and recruitment.

Recent work has suggested that microbiota-directed epigenomics is a central mechanism driving intestinal development [74,75]. Genome-wide examination of the postnatal methylome revealed microbiota-dependent increases in DNA methylation in intestinal stem cells within maturation-associated genes that positively correlated with increased gene expression [76]. IEC-specific deletion of the methyltransferase Dnmt1 resulted in global DNA hypomethylation, aberrant crypt formation and stunted colonic development [76,77]. While SCFAs and the epigenome have not been studied *in vivo* to the same extent as immune cells, recent work has demonstrated previously unrecognized differential effects of butyrate on differentiated versus stem cell IECs (Fig. 2). Specifically, more superficial colonocytes lining the crypt metabolize bacterial-derived butyrate as a preferred energy source, preventing butyrate from diffusing to the crypt base where IEC stem cells reside [78,79]. When exposed to butyrate, intestinal stem cells exhibited increased levels of histone acetylation, along with impaired epithelial proliferation and repair [79].

Within the host IECs, initial studies have supported that expression of HDAC epigenomic-modifying enzymes mediate microbiota-dependent intestinal homeostasis. For instance, deletion of HDAC3 in IECs increased in H3K9 acetylation at upregulated genes, and led to altered IEC homeostasis and impaired intestinal barrier function [80]. However, loss of HDAC3 in IECs of germ-free mice minimally impacted gene expression and phenotype, suggesting that HDAC3 mediates integration of microbiota-derived signals that maintain healthy intestinal homeostasis. Other class I HDACs, HDAC1 and HDAC2, were also found to be essential for maintaining IEC lineages, barrier function and protection from damage-induced colitis, albeit these effects were not studied in the relation to microbiota dependence [81–83]. Collectively, these studies highlight that regulation of epigenetic modifying enzymes, such as HDACs, represents a new mechanistic link for future investigation into how the microbiota may directly trigger epigenomic regulation in IECs.

Microbial-derived metabolites shape the epigenomics of the mammalian nervous system

Increasing evidence highlight the importance of gut microbiota in the development and function of both the enteric nervous system (ENS) and central nervous system (CNS) [84]. Cells of the ENS that innervate the intestine respond to microbiota-derived signals as germ-free mice display decreased enteric neurons, overall neuron excitability and gut motility [85,86]. SCFAs are known to regulate hormone and neurotransmitter production as well as colonic motility [87–89]. Further, *in vitro* treatment of an enteric glial cell line with butyrate resulted in increased H3K9 acetylation [90], supporting the hypothesis that butyrate induces epigenetic modifications in the ENS. There is also increasing appreciation for bidirectional communication between gut microbiota and the CNS, as dysbiosis has been implicated in altered stress, autism, pain, and memory [7]. SCFAs can cross the blood-brain barrier and therefore represent a mechanism by which the intestinal microbiota may also influence the CNS. A crucial role for the gut microbiota and SCFAs was demonstrated in the development, organization and function of microglia, a cell-lineage referred to as the macrophage of the brain [91]. Microglia of germ-free mice exhibited reduced expression of genes involved in cell activation and type I interferon signaling. Interestingly, expression of

several genes encoding proteins related to histone acetylation and histone methylation (i.e. HDAC1 and Kdm6b) were altered in germ-free microglia, and administration of SCFAs to germ-free mice partially rescued microglial dysfunction. Although further studies are needed to test how epigenomic pathways mediate crosstalk between the microbiota the CNS and ENS, these recent findings suggest that intersection of the microbiota and host epigenomics represents a potentially profound level of regulation in the nervous system.

Conclusion and Perspectives

Collectively, recent work has revealed substantial ties between the microbiota and epigenomic regulation in mammalian cells. Notably, microbiota-dependent immune cell polarization through epigenomic mechanisms demonstrates a key role for the microbiota in preventing mucosal inflammation, while simultaneously enhancing barrier function against pathogens through IEC-intrinsic processes. As such, diseases associated with mucosal inflammation, impaired barrier function, and dysbiosis may result from dysregulation of the epigenomic crosstalk between the host and microbiota that is necessary to maintain healthy intestinal symbiosis.

While these recent discoveries are certainly uncovering new approaches to consider when examining host-microbiota interactions, our understanding of how epigenomic modifications are involved in mediating these interactions is only starting to be revealed. SCFAs in particular seem to be a critical subset of microbiota-derived metabolites with potential to modulate the epigenomic landscape and transcriptional output. However, it remains unclear which mechanisms (GPR signaling, HDAC inhibition or a combination) primarily mediate SCFA effects on the epigenome. The microbiota is remarkably diverse with effects on mammalian cells that are independent of SCFAs. Therefore, further exploration into host mechanisms integrating microbiota-derived cues, and specific microbiota-derived signals that induce epigenomic change, will be key for deepening our understanding of complex host-microbiota interactions in health and disease. Deciphering microbiota-directed epigenomic pathways could also guide novel potential therapeutic approaches against microbe-influenced diseases.

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Highlights

- Epigenomic modifications modify transcription in response to environmental cues.
- Epigenomic regulation of intestinal homeostasis can be altered by the microbiota.
- Microbiota-derived short chain fatty acids inhibit histone deacetylases.
- Epigenomics represents a developing link between the microbiota and host cells.

Figure 1. Epigenomic regulation of immune homeostasis by the intestinal microbiota

The intestinal microbiota and microbiota-derived metabolites such as short-chain fatty acids (SCFAs) regulate the epigenome of various hematopoietic cell types. (1) Macrophages and dendritic cells can sense SCFAs in part through G-protein-coupled-receptors (GPCRs). This regulation correlates with increased global histone H3 acetylation, expression of anti-inflammatory cytokines, retinoic acid (RA) signaling and regulation of regulatory T cells (Tregs) [33–39]. (2) SCFAs butyrate and propionate promote Treg generation through activation of GPR43 signaling and suppression of HDAC activity. This results in increased histone acetylation in the *Foxp3* gene and increased *Foxp3* expression [35,59,61]. (3) The microbiota suppresses the generation of invariant natural killer cells (iNKTs) by reducing *Cxcl16* 5' CpG methylation and reducing *Cxcl16* expression [57]. (4) Microbiota may influence intestinal innate lymphoid cell (ILC) homeostasis through epigenomic modifications [41,42].

Figure 2. Microbiota-dependent regulation of epigenomic pathways in intestinal epithelial cells
 The microbiota provide signals to the intestinal epithelium that contribute to effective intestinal barrier function. (1) Neonatal exposure to a complex microbiota establishes CpG methylation patterning in intestinal stem cells that are linked to stem cell renewal and IEC differentiation [76]. Short chain fatty acids (SCFAs) are taken up as energy by superficial colonocytes lining intestinal crypts and thus prevent diffusion to the stem cell niche where these metabolites inhibit stem cell proliferation [79]. (2) Interactions between IECs and the microbiota involve regulation of histone deacetylases (HDACs) to modify the host epigenome and influence gene expression and barrier functions [80]. HDAC expression in IECs maintains intestinal homeostasis and barrier integrity [80–83].