Epigenetic regulation by gut microbiota

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ABSTRACT

The gastrointestinal tract is continuously exposed to trillions of commensal microbes, collectively termed the microbiota, which are environmental stimuli that can direct health and disease within the host. In addition to well-established bacterial sensing pathways, microbial signals are also integrated through epigenetic modifications that calibrate the transcriptional program of host cells without altering the underlying genetic code. Microbiota-sensitive epigenetic changes include modifications to the DNA or histones, as well as regulation of non-coding RNAs. While microbiota-sensitive epigenetic mechanisms have been described in both local intestinal cells and as well in peripheral tissues, further research is required to fully decipher the complex relationship between the host and microbiota. This Review highlights current understandings of epigenetic regulation by gut microbiota and important implications of these findings in guiding therapeutic approaches to prevent or combat diseases driven by impaired microbiota-host interactions.

Introduction

The mammalian gastrointestinal tract is a complex ecosystem that is densely populated by trillions of commensal bacteria, fungi, archaea, and viruses, collectively termed the microbiota. Research from recent decades has highlighted the importance of the microbiota in the development and maintenance of normal intestinal development and physiology, including digestion and nutrient uptake, metabolism, tissue development, and immunity. Importantly, though, changes in the composition or abundance of the microbiota have been associated with chronic human conditions such as inflammatory bowel disease (IBD), metabolic disorders and cancer. Commensal microbes interact closely with intestinal epithelial cells (IECs) which exist at the direct interface between the microbiota and underlying host cells, and are therefore optimally poised to respond to resident microbes and their metabolites. Microbial signals are equally important in the development and maintenance of innate and adaptive immune cells. In addition to regulating local intestinal cells, the microbiota can also impact peripheral tissues through systemic trafficking of microbial components and microbiota-derived metabolites. The combined abundance and proximity of commensal microbes to mammalian cells emphasize the microbiota as a large source of environmental stimuli that can shape host cellular function. In support of this notion, epigenetic regulation by the microbiota has emerged as a fundamental interface by which commensal microbes dynamically influence intestinal biology.

Epigenetic regulation linking the microbiota and mammalian cells

Epigenetic modifications enable mammalian cells to alter gene expression without modifying the genetic code, and, therefore, represent a fundamental mechanism by which mammalian cells can adapt their transcriptional program to environmental cues. In eukaryotic cells, genetic information encoded within the DNA is packaged around histone proteins and organized into a compact structure called chromatin. In general, epigenetic modifications that promote relaxation of the chromatin (euchromatin) are associated with active gene transcription, whereas condensation of...
the histone-DNA complexes (heterochromatin) denote inaccessible and silenced regions. Epigenetic modifications that dynamically regulate chromatin accessibility include post-translational modification of histones and DNA methylation, which are controlled by the activity of opposing epigenetic modifying enzymes. Furthermore, non-covalent epigenetic mechanisms that control gene expression, such as micro-RNAs (miRNAs) and long-noncoding RNAs (lncRNA), have also been implicated in initiating and sustaining epigenetic change. Together, these epigenetic mechanisms influence the cellular transcriptional program by directing accessibility of histone modifiers, transcription factors and transcriptional/translational machinery to the DNA or RNA transcripts. Epigenetic regulation is being increasingly recognized as a potent mechanism through which the microbiota influence host physiology and can occur through multiple potential mechanisms: 1) microbial biosynthesis or metabolism that influence the availability of chemical donors for DNA or histone modifications; 2) regulation of epigenetic modifying enzyme expression and/or activity; or 3) activation of host-cell intrinsic processes that direct epigenetic pathways.

Microbiota-derived metabolites as epigenetic substrates and enzymatic regulators

Epigenetic-modifying enzymes require appropriate substrates to catalyze changes to the chromatin. For instance, DNA/histone methyltransferases (DNMTs/HMTs) and histone acetyltransferases (HATs) generally rely on methyl and acetyl donors, respectively, for their catalytic activity. While many of these donor substrates can be generated from host-intrinsic pathways, the microbiota is being increasingly appreciated as an additional source of these molecules. Indeed, the microbiota can synthesize a plethora of biological compounds including several that serve as epigenetic substrates, co-factors or regulators of epigenetic enzyme activity. In particular, the intestinal microbiota is known to generate folate and other B vitamins (B2, B12) that donate methyl groups for DNA or histone methylation (Figure 1). Folate is an essential nutrient produced by numerous commensal microbes including probiotic *Bifidobacterium* and *Lactobacillus* species that participate in one-carbon metabolism to generate S-adenosylmethionine (SAM), the primary substrate for DNA and histone methylation. Commensal microbes can also metabolize dietary methionine into SAM. Thus,
changes in bacterial composition can influence SAM availability and correspondingly shift the DNA or histone methylation status in the host.

Short-chain fatty acids (SCFAs) (discussed in more detail below) represent another important group of epigenetically relevant molecules that are exclusively produced by commensal microbes through fermentation of complex non-digestible carbohydrates and fiber. Importantly, SCFAs inhibit deacetylase activity of histone deacetylases (HDACs), resulting in chromatin changes generally associated with increased expression of target genes (Figure 1). Germ-free (GF) mice possess minimal levels of both intestinal and peripheral SCFAs,17–19 and supplementation of SCFAs in GF mice can largely restore transcriptional and epigenetic changes instilled by the microbiota.20 Concurrent with HDAC inhibition, SCFAs also contribute to cellular acetyl coenzyme A levels (acetyl donor), which subsequently affects other TCA intermediates such as a-ketoglutarate, fumarate, and succinate that modulate enzymatic activity of ten-eleven translocation (TET) methylcytosine dioxygenases that are involved in DNA methylation.21

**Microbiota direct DNA methylation patterns during homeostasis and inflammation**

DNA methylation is an epigenetic modification whereby methyl groups (-CH₃) are covalently added to cytosine or adenine bases by DNA methyltransferases (DNMTs) using the donor metabolite SAM. Methylated DNA residues are often located within CpG Islands and are generally associated with gene repression by physically restricting access of regulatory mediators to the DNA, especially when located near gene promoters. Changes in DNA methylation were initial evidence that the microbiota influences the host epigenome. Specifically, DNA methylation of the 5’ CpG Island in the toll-like receptor 4 (Tlr4) gene was decreased in large intestinal IECs from GF mice compared to conventionally housed (CNV) mice, resulting in reduced Tlr4 expression and responsiveness to the pathogen-associated molecular pattern lipopolysaccharide (LPS).22 Demethylation was also detected at the chemokine ligand Cxcl16 gene in the colon and lungs of GF mice compared to CNV mice, which is consistent with decreased mucosal invariant natural killer T cell accumulation in absence of the microbiota.23 Microbiota also directed DNA methylation in regulatory T cells (Tregs) by increasing expression of Uhrf1, a DNA methylation adaption protein that complexes with Dnmt1 and HDAC1.24 T-cell specific deletion of Uhrf1 impaired normal cell cycle gene expression in Tregs and consequently led to spontaneous colitis.

Subsequent studies have since discovered that the microbiota more broadly impacts the mammalian DNA methylome in multiple cell types and tissues during homeostasis and disease. Transcriptome and global DNA methylation analyses on colonic IECs from GF and CNV mice revealed that microbiota-exposure induced DNA hypomethylation and increased expression of anti-bacterial and anti-inflammatory genes.25 This microbiota-dependent demethylation corresponded with increased expression and activity of the DNA demethylase enzymes Tet3 and Dnmt1 in CNV IECs,25,26 and IEC-specific Tet3 deletion led to an increase in global DNA methylation including microbiota-sensitive regions.26 During chemically induced dextran sulfate sodium (DSS) colitis, CNV IECs undergo further hypomethylation, whereas GF mice remain relatively unchanged.25 In humans, DNA methylation in colonic biopsies correlated with microbiota composition, inflammation status, and disease classification of ulcerative colitis (UC) and Crohn’s disease patients.27 UC has been shown to precede the development of colorectal cancer in certain patients, and disease progression has been associated with microbial dysbiosis.28,29 Notably, high *Fusobacterium* levels were associated with increased DNA methylation at colorectal-cancer related genes in UC patients.30 Colorectal cancer-associated microbiota further correlated with DNA methylation in a large patient cohort and, when colonized into GF mice, promoted epithelial hyperplasia and DNA methylation in the intestine.31 The link between intestinal microbial composition and DNA methylation was also observed in obese human subjects, especially at genes involved in energy regulation in the blood and adipose tissue.32 Collectively, these findings highlight the relationship between microbiota and DNA
methylation signatures under homeostatic and pathologic conditions, and that microbiota-associated DNA methylation changes may predict inflammation-driven carcinogenesis in the intestine.

In addition to microbiota-dependent DNA methylation in mammalian cells in adults, recent studies have emphasized the importance of DNA methylation induced by commensal microbes during early life. The neonatal intestine is uniquely sensitive to initial microbial colonization after birth, with the complexity and density of the microbiota continuously expanding into adulthood. This critical early window is important for post-natal tissue development and establishing normal immune responses. Microbiota-sensitive differential DNA methylation and gene expression were detected in mice small intestinal IECs during postnatal development and correlated with shifts in transcript levels of DNA methylation/demethylation enzymes Dnmt3a and Tet3. Early microbial colonization was also associated with altered DNA methylation in genes associated with immunity, metabolism, and vascular regulation in ileal IECs from CNV and antibiotic-treated preterm piglets. Similar changes were demonstrated in intestinal stem cells in the colon, where developmentally established DNA methylation patterns, particularly at the 3’ CpG Islands of glycosylation genes involved in cellular maturation, were substantially blunted in the absence of gut microbiota. Further, this was not due to impaired Dnmt1 activity, contrary to findings in mature IECs. Neonatal colonization also protected against pulmonary and enteric disease by preventing accumulation of invariant natural killer T cells in lungs and intestines of mice by decreasing Cxcl16 DNA methylation and gene expression in those cells. In vitro, differential DNA methylation was observed in human fetal and mature intestinal epithelial cell lines exposed to commensal or pathogenic bacteria, and fetal cells were more sensitive to pathogen-induced modifications compared to adult. These findings emphasize the importance of microbiota-epigenome cross-talk during early life stages, and that timing, density, and diversity of microbial colonization likely influence this interaction into adulthood.

### Host–microbiota interactions through histone modifications

Post-translational modifications of histone proteins represent another central mechanism for microbial regulation of host chromatin. Instead of targeting DNA directly, these types of modifications are typically covalently added to lysine [K] residues on histone tails to influence chromatin conformation and gene expression. While over 20 unique classes of histone modifications have been identified, histone acetylation and methylation have been most commonly studied in context of microbial regulation. These modifications are balanced by the activity of opposing classes of epigenetic enzymes (i.e. HATs versus HDACs; histone methyltransferases (HMTs) versus demethylases (HDMs)). Importantly, though, new studies are carving out potential roles for additional histone modifications, such as crotonylation and ethylation, in facilitating crosstalk between the microbiota and host.

Changes in chromatin accessibility as a result of microbiota-induced histone modifications has been described in multiple tissues and cell populations. For instance, gut microbiota was found to globally alter histone H3 and H4 acetylation and methylation in various tissues (colon, liver, and adipose tissue) in a diet-dependent manner, and supplementation of SCFAs to GF mice partially restored microbiota-sensitive histone modifications and gene expression. A recent study also showed that cecal microbiota transfer from dams to pups exposed to antibiotics early in life restored histone modifications in the ileum and liver. Specifically, this microbiota-transfer led to lysine 27 (K27) deacetylation and trimethylation, potentially through enhanced Jumonji family-related lysine demethylase D3 (Jmjd3/Kdm6b) activity in response to Tlr2 induction, indicating that microbiota prevents expression of antibiotic-induced genes. In the intestinal epithelium, differential H3K4me3, a histone methylation associated with active transcription, was also identified at genes in ileal IECs from GF compared to CNV mice, a subset of which overlapped with H3K4me3 profiles unique to newly diagnosed IBD patients compared to healthy controls. This modification was also found to be regulated specifically at Wnt-responsive gene
promoters by microbiota-derived gallic acid in a colorectal cancer mouse model, supporting that the microbiota not only interact with host chromatin through histone modifications at steady-state, but also in context of inflammation and disease.

Consistent with global changes in histone acetylation driven by the microbiota, studies have demonstrated that HDAC enzymes are involved in maintaining microbiota-dependent intestinal homeostasis. In particular, HDAC3, a class I histone deacetylase that is highly expressed in the intestinal epithelium, is sensitive to microbial signals. IEC-specific deletion of HDAC3 (HDAC3\textsuperscript{ΔIEC}) increased IEC-H3K9 acetylation and gene expression, altered Paneth cell homeostasis and intestinal barrier function, and worsened response to DSS-induced intestinal inflammation.\textsuperscript{47} Importantly, abnormalities in HDAC3\textsuperscript{ΔIEC} mice were only evident in CNN mice, and not in GF mice, indicating that HDAC3 is essential to maintain normal commensal-host relationships. In context of a high-fat diet, alterations in microbiota-derived signals and disruption of intestinal epithelial HDAC3 prevented the development of obesity.\textsuperscript{48,49} Consistent with these studies, increased enrichment of H3K27Ac, a marker of active enhancers, was described at metabolic genes in colonic IECs in response to obesity-associated microbiota transfer compared to control microbiota recipients.\textsuperscript{50} Microbiota also improved defense against the bacterial pathogen \textit{Citrobacter rodentium} through microbiota-dependent regulation of IEC-intrinsic HDAC3.\textsuperscript{51,52}

The most abundant SCFAs in the mammalian intestinal lumen include acetate, propionate, and butyrate, which are found at molar ratios of 60:20:20 in the intestinal tract and can reach peak concentrations of up to 100 mM.\textsuperscript{53} SCFAs inhibit HDACs, often resulting in increased histone acetylation at direct targets (Figure 1). Despite the abundance of HDAC-inhibiting SCFAs in the gut, recent work found that HDAC3 activity was elevated in IECs from CNN mice compared to GF mice due to production of an HDAC-activating metabolite, inositol triphosphate (IP3), by intestinal commensal bacteria.\textsuperscript{54} Consistent with increased HDAC activity, IECs from mice mono-colonized with IP3-producing bacteria exhibited decreased H3K9Ac at HDAC3 target genes relative to mice with lower IP3 in the intestine. In intestinal epithelial stem cells, which reside at the base of the crypts and are anatomically protected from SCFA exposure, administration of butyrate directly to these cells inhibited HDAC activity and increased H3K9 and K27 acetylation, which coincided with impaired proliferation and response to injury.\textsuperscript{55} Thus, HDAC3 regulation by the microbiota occurs in multiple epithelial cell populations and reflects more than SCFA-mediated inhibition alone, and instead relies on integration of multiple microbiota-derived signals. Additional studies are needed to determine whether other histone modifiers known to control intestinal physiology, such as HDAC1 and HDAC2,\textsuperscript{56–58} are similarly regulated by the microbiota.

Lysine crotonylation is a relatively less studied histone modification that is often enriched at active promoters and enhancers in mammalian cells.\textsuperscript{59} While the addition or removal of crotonyl motifs can be catalyzed by specialized histone crotonyl-transferases and decrotonylases, HATs and HDACs have also been reported to exhibit histone crotonyl-modifying activity.\textsuperscript{60} Consistent with this finding, Histone H3 and H4 crotonylation levels (H3K18Cr, H3K8Cr, and H4K8Cr) were significantly decreased in the colon of mice treated with antibiotics and administering the HDAC inhibitor butyrate promoted histone crotonylation.\textsuperscript{42} Taken together, these studies demonstrate that the microbiota stimulate multiple types of histone modifications and regulate activity of histone-modifying enzymes to calibrate local and extra-intestinal chromatin landscapes.

Immune cells similarly undergo broad histone modifications in response to microbial colonization. Non-mucosal mononuclear phagocytes (macrophages and dendritic cells) require microbial signals to epigenetically and transcriptionally activate genes involved in interferon (IFN) signaling and initiating normal T cell responses.\textsuperscript{61,62} Specifically, GF or antibiotic-treated mice have decreased H3K4me3 (activating) at genes involved in pro-inflammatory responses including type I IFNs and increased H3K27me3 (repressive) at metabolic pathways compared to microbiota-exposed cells. The microbiota further maintain this anti-inflammatory phenotype in macrophages
through HDAC3-mediated histone deacetylation of the pro-inflammatory cytokine IL-12β.\textsuperscript{53} Similar changes in chromatin accessibility defined by differential H3K4me2 enrichment were shown at lineage-defining regulatory elements in innate lymphoid cells (ILCs) to favor an ILC3 phenotype over ILC1 or ILC2 in response to the microbiota.\textsuperscript{64} Ethionine was recently discovered as a novel microbiota-derived metabolite produced by the commensal bacterium \textit{Lactobacillus reuteri} through a 2-carbon folate cycle.\textsuperscript{43} In agreement with previous reports that ethionine inhibits histone methylation,\textsuperscript{65} mass spectrometric analyses revealed that human monocytic THP-1 cells treated with ethionine preferentially incorporated ethyl groups into lysine residues of histone H3 (K9/K10/K26) instead of methyl groups. Moreover, ethionine-treated monocytes failed to activate NF-kB signaling or TNF-α expression in response to LPS treatment.\textsuperscript{43}

Similar to IECs, distinct bacterial populations have also been shown to modulate histone modifications in immune cells. Colonization with the commensal bacteria segmented filamentous bacteria (SFB) or administration of SFB-induced serum amyloid A (SAA) increased expression of the demethylase Jmd3 and increased global H3K27me3 in bone marrow-derived cells, resulting in improved protection against \textit{Entamoeba histolytica} infection.\textsuperscript{66} \textit{Clostridium scindens} colonization in mice promoted expression of the same H3K27-targeting demethylase in bone marrow-derived granulocyte-macrophage progenitors (GMPs) and increased H3K4me3 (activating) and decreased H3K27Ac (repressive) in the promotor regions of granulopoiesis genes C/EBPA and C/EBPB, respectively, leading to increased neutrophils and enhanced protection against \textit{E. histolytica} infection.\textsuperscript{67} \textit{C. scindens} was also demonstrated to metabolize the bile salt cholate into deoxycholate, and administration of deoxycholate increased Jmd3 expression in GMPs.\textsuperscript{67} Collectively, these studies demonstrate the intestinal microbiota or individual commensal bacterial populations alter the chromatin landscape in multiple immune cell types by directing covalent modifications of histones or regulating the activity of histone-modifying enzymes.

In addition to global changes in the histone landscape at steady state, recent insights reveal that the microbiota exhibits a circadian rhythmicity that programs the host transcriptome and epigenome at the histone modification level.\textsuperscript{49,68} Diurnal cycles in microbiota composition and abundance that occurred throughout the day in mice corresponded to similar rhythmicity in histone modifications marking active promoters (H3K27Ac and H3K4me3), enhancer activity (H3K27Ac and H3K4me2), and gene expression of circadian genes in colonic IECs. Oscillations in chromatin landscape and transcriptional responses were largely lost upon microbiota-depletion using antibiotics or comparing GF mice, indicating that normal oscillations of the microbiota and microbiota-mediated control of the circadian transcriptome is necessary to maintain normal circadian cycles.\textsuperscript{68} Similar cycling patterns in H3K27 and K9 acetylation were detected in small intestines of CNV, but not GF mice that was attributed to a lack of oscillating expression of the histone deacetylase HDAC3 in absence of commensal microbes.\textsuperscript{49} Interestingly, mono-colonization of GF mice with \textit{Bacteroides thetaiotamicron} was sufficient to increase HDAC3 expression,\textsuperscript{49} re-iterating that specific commensal species can have potent effects on the host epigenome, and that the natural circadian rhythmicity of the microbiota adds an additional layer of complexity in this regulation.

**Regulation of immune cells by SCFAs**

SCFAs have become well-appreciated intermediary between the microbiota and mucosal immune cell populations. Importantly, regulation by SCFAs extends to innate and adaptive immune compartments locally in the intestine as well as systemically. This has been described to occur through a combination of HDAC inhibition and G-protein coupled receptor (GPR) activation.\textsuperscript{69,70} Treatment of intestinal or bone marrow-derived macrophages with butyrate inhibited HDAC activity and increased histone H3 acetylation at secondary response genes including IL-6, IL-12, and mediators of STAT6 signaling.\textsuperscript{19,71} However, in contrast to the predicted inhibitory effect of SCFAs on HDACs, expression of these same genes were
instead decreased in response to butyrate, reflecting an anti-inflammatory M2 macrophage state that attenuated intestinal inflammation in response to DSS-induced colitis.\textsuperscript{19,71} Furthermore, butyrate, and to a lesser degree propionate, improved defense against enteric pathogens by promoting monocyte to macrophage differentiation and activating an antimicrobial transcriptional program through HDAC3 inhibition.\textsuperscript{72} SCFAs also promoted IL-22 histone acetylation and production by ILCs and CD4\textsuperscript{+} T cells through a combination of GPR41 activation and HDAC inhibition, resulting in enhanced protection from intestinal inflammation during bacterial infection.\textsuperscript{73} Butyrate was further shown to inhibit HDACs and activate GPR109A in colonic macrophages and dendritic cells, enabling them to promote anti-inflammatory regulatory T cells (Tregs).\textsuperscript{18,74,75} Thus, SCFAs control epigenetic mechanisms and transcriptional responses in immune cells to support an overall anti-inflammatory phenotype during chemically induced colitis and pathogenic infection.

Tregs are defined by their expression of the transcription factor Foxp3 and act to suppress aberrant inflammatory and effector responses, thereby playing a critical role in immune homeostasis and maintaining tolerance to the microbiota. Colonization with Clostridial species, or treatment with Clostridia-derived butyrate, increased the frequency of circulating Tregs by inducing acetylation on histone H3 within regulatory regions of the Foxp3 locus.\textsuperscript{17} Post-transcriptional acetylation of Foxp3 proteins also occurred in peripheral CD4\textsuperscript{+} T cells, thereby enhancing expression and protein stability.\textsuperscript{18} In agreement with these findings, treatment with the SCFA propionate, or deletion of HDAC3, HDAC6, or HDAC9 in mice resulted in both increased H3K9 acetylation at the Foxp3 locus and enhanced Foxp3 protein stability.\textsuperscript{76–78} Acetate, another microbiota-derived SCFA, also inhibited HDAC activity and increased overall acetylation of histones H3 (K9) and H4 (K5/8/16) in CD3/CD28 activated primary human CD4\textsuperscript{+} T cells,\textsuperscript{79} although HDAC inhibition by acetate is somewhat controversial.\textsuperscript{18,80,81}

In contrast to physiological concentrations of butyrate, elevated butyrate levels induced expression and H3 acetylation of Th1-associated genes including IFN\textgamma and Tbx21 (encodes T-bet) in T cells under Treg-inducing conditions, while expression of Th17- and Th2-promoting transcription factors ROR\gammat and Gata3, respectively, were not induced.\textsuperscript{82} Moreover, higher concentrations of butyrate administered to GF mice resulted in elevated IFN\textgamma production and exacerbated disease during DSS-induced colitis. Another study similarly found that butyrate promoted Th1, but suppressed Th17 polarization from naïve T cells in culture and \textit{in vivo} by inhibiting HDAC activity independently from GPR43 activation to induce expression of T-bet and the anti-inflammatory cytokine IL-10.\textsuperscript{83} Congruent with enhanced IL-10 in response to butyrate treatment of bulk CD4\textsuperscript{+} T cells, microbiota-specific T cells isolated from butyrate-treated CBir1 transgenic mice caused less severe colitis in Rag\textsuperscript{−/−} mice compared to control T cells.\textsuperscript{83,84} \textit{Faecalibacterium prausnitzii}-derived butyrate similarly suppressed Th17 differentiation and simultaneously promoted Tregs by inhibiting HDAC3 and HDAC1 deacetylase activity \textit{in vitro} and \textit{in vivo}.\textsuperscript{85,86} This is similar to HDAC3 inhibition in IECs in response to \textit{F. prausnitzii} colonization.\textsuperscript{54} Besides butyrate, Pentanoate, a 5-carbon SCFA, also reduced IL-17A production by CD4\textsuperscript{+} T cells through HDACs, and induced IL-10 gene histone acetylation and expression in CD4\textsuperscript{+} T cells and regulatory B cells, thereby dampening T-cell mediated autoimmune responses.\textsuperscript{87} Thus, SCFAs exert context-and concentration-dependent effects that generally promote a low Th17/high Treg phenotype that is protective against intestinal inflammation.

CD8\textsuperscript{+} cytotoxic T lymphocytes (CTLs) defend against intracellular pathogens and possess cancer-targeting abilities. Recent findings demonstrate that butyrate, and to some degree propionate, increased effector molecule expression including CD25, IFN\textgamma and TNF-\alpha in splenic and mesenteric CD8\textsuperscript{+} T cells independently from GPR41 or GPR43 activation, resulting in enhanced anti-tumor activity.\textsuperscript{88} Instead, this regulation was driven by HDAC inhibition with corresponding increases in histone H4 acetylation directly at the promoters of IFN\textgamma and Tbx21, a similar result described for CD4\textsuperscript{+} T cells exposed to butyrate under Treg, Th1- or Th2-polarizing conditions.\textsuperscript{82,89} It was subsequently also discovered that butyrate, and \textit{Megasphaera massiliensis}-derived pentanoate, improved the anti-
tumor reactivity of antigen-specific CD8+ T cells by increasing their production of effector cytokines IFNγ and TNF-α through HDAC inhibition.90 Additionally, studies showed that increased SCFAs from high-fiber feeding in mice encouraged CD8+ T cell activation and later transition into memory cells in context of viral infections.91,92 Microbiota-derived SCFAs also supported intestinal B cell differentiation and antibody production by inhibiting HDAC activity leading to increased histone acetylation (H3K9Ac) and gene expression of multiple genes associated with B cell function.93 Similar effects were observed in human and mouse B cells treated specifically with butyrate and propionate that dose-dependently counteracted HDAC-mediated H3K9 deacetylation and transcriptional repression of miRNAs that target genes involved in B cell differentiation.94 Furthermore, butyrate and propionate restricted normal B cell intrinsic functions including immunoglobulin class switching and somatic hypermutation. Thus, microbiota-derived SCFAs have potent immunomodulatory effects on both innate and adaptive immune cells in the host that actively maintain homeostasis and dampen inflammation in the intestine.

Non-covalent modifications

Epigenetic regulation by non-coding RNAs (ncRNAs), which are RNA molecules that do not encode a functional protein, has recently been linked to microbiota–host interactions. ncRNAs are functionally categorized based on nucleotide length, and recent studies have shown that ncRNAs can regulate gene expression at the gene or chromosome level. Long non-coding RNAs (lncRNAs) typically contain over 200 nucleotides and regulate gene expression by acting directly as DNA scaffolding to modify chromatin interactions or by complexing with chromatin modifiers and/or transcriptional machinery.95 Initial investigation into commensal-regulated lncRNAs focused on characterizing these transcripts in intestinal epithelial tissues from GF, CNV, or GF mice mono-colonized with E. coli or E. coli expressing a bile salt hydrolase.96 These analyses identified hundreds of lncRNA differentially regulated between IECs from GF and CNV mice. Interestingly, expression of only six lncRNAs overlapped between the different colonizations, and lncRNA signatures were able to distinguish mice with unique microbial compositions, demonstrating a degree of commensal species-specificity in this regulation. Microbiota-induced lncRNAs and were also found to be highly expressed in the spleen and thymus, suggesting that microbiota-dependent lncRNAs may be involved in host immune regulation.96 Global transcriptional profiling of lncRNAs in GF and CNV mice further revealed tissue-specific modulation of lncRNAs in the intestine and peripheral tissues in response to the microbiota.97 Furthermore, predicted interactions between lncRNAs and target protein-coding RNAs were functionally enriched for genes related to nutrient absorption and metabolic pathways across different tissues. Thus, the microbiota not only regulates expression of lncRNAs in the intestine and immune organs, but also remotely in metabolic tissues. Additional studies are needed to further elucidate the tissue-specific and functional relevance of microbiota-sensitive lncRNAs and mechanisms underlying their regulation.

miRNAs are short, single-stranded ncRNAs (~22 nucleotides) that are abundantly expressed in mammalian cells and can also circulate systemically.98 Unlike lncRNAs that target the chromatin, miRNAs primarily interfere with post-transcriptional gene expression by base-pairing with complementary protein-coding mRNA sequences. miRNAs can also occasionally mediate DNA methylation or histone modifications at gene promoters to influence target gene expression,99,100 however this has yet to be explored in context of the microbiota. miR-181a and miR-181b, two miRNAs reported to control inflammation- and obesity-associated pathologies, were recently found to be significantly upregulated in epididymal white adipose tissue from specific pathogen-free (SPF) mice as compared to GF mice.101 It was also recently shown that transfer of maternal cecal contents into antibiotic treated pups restored global ileal epithelial miRNA expression profiles including miRNAs that target microbiota-sensitive genes involved in regulating innate and adaptive immunity such as CD44, Tlr2, and Reg3g.44 Taken together, these studies indicate that further investigation into miRNA regulation by the microbiota has the
potential to expand our understanding of microbiota-induced control of host physiology and disease beyond epigenetic regulation of histones and the DNA.

**Conclusions and future perspectives**

Recent research has highlighted that epigenetic modification of the host by commensal microbes represents a broad and fundamental level of regulation during early life development, homeostasis, and disease. Indeed, studies have shown that host cells integrate beneficial microbial cues that mediate these processes through epigenetic pathways, and that perturbation of the microbiota–host relationship associated with pathologies, including IBD and colorectal cancer, correlate with altered epigenetic patterning. However, our mechanistic understanding of microbiota–host epigenome interactions underlying this regulation is still in need of deeper investigation.

While much emphasis has been placed on SCFAs, implementation of multi-omics approaches has revealed that the microbiota is responsible for the production of many more biologically active metabolites that may facilitate epigenetic modification. Future work will determine the involvement of more recently identified microbiota-derived molecules on the host epigenome. Furthermore, comparative metagenomic microbial analyses in humans and the discovery of commensal species-specific epigenetic modifications indicate that enrichment for certain microbial species has the potential to modulate unique gene expression signatures. Recent associations between microbiota composition and epigenetic modifications in IBD, cancer, and obesity also indicate that epigenetic patterns could serve as diagnostic tools to link genetic predisposition and microbial dysbiosis with disease pathogenesis. Improved chromat analytical techniques, including the ability to examine epigenetic marks on single cells and limited cell numbers could reveal epigenetic heterogeneity within a cell population or in rare cell types in response to microbiota. Finally, genetic manipulation of epigenetic-modifying bacteria or controlling microbial-produced epigenetic substrate levels could represent a new avenue for pre- or probiotic approaches to provide a more targeted, localized method to control epigenetic enzymes in the intestine.

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