Chemical signaling between gut microbiota and host chromatin: What is your gut really saying?

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Mammals and their gut microbial communities share extensive and tightly coordinated co-metabolism of dietary substrates. A large number of microbial metabolites have been detected in host circulation and tissues and, in many cases, are linked to host metabolic, developmental, and immunological states. The presence of these metabolites in host tissues interacts with regulation of the host’s epigenetic machinery. Although it is established that the host’s epigenetic machinery is sensitive to levels of endogenous metabolites, the roles for microbial metabolites in epigenetic regulation are just beginning to be elucidated. This review focuses on eukaryotic chromatin regulation by endogenous and gut microbial metabolites and how these regulatory events may impact host developmental and metabolic phenotypes.

The eukaryotic genome exists in a largely static state, yet gene expression patterns are remarkably plastic in response to environmental stimuli. This adaptability is governed by epigenetic mechanisms that alter chromatin structure through a combination of covalent post-translational modifications (PTMs)4 of histone proteins, histone variant deposition, DNA methylation, and noncoding RNAs. Furthermore, these programming events can result in either transient or more long-term, even transgenerational, effects. For example, histone acetylation has been demonstrated to have a half-life of 53–87 minutes, depending on the specific lysine residue being modified, which is considerably faster than a typical mammalian cell cycle (1). In contrast, parental and early life nutritional status has been shown to elicit persistent effects on the DNA methylomes of offspring. Natural variation in dietary intake of methyl-donor nutrients in rural Gambian mothers, which associated with seasonal differences in maternal plasma biomarker concentrations, enabled prediction of infant DNA methylation patterns based on the season at the time of conception (2). In mice, suboptimal nutrition during fetal development affected the DNA methylome of male F1 offspring and was transmitted through the paternal line to F2 offspring (3). Thus, environmental factors can “program” chromatin states and drive both short- and long-term effects.

The molecular machinery responsible for depositing and removing histone and DNA modifications is known to be sensitive to the availability of small molecule metabolites, many of which serve as co-substrates in these enzyme-catalyzed transformations. For example, histone acetyltransferases (HATs) require sufficient availability of acetyl coenzyme A (acetyl-CoA), a key metabolite at the intersection of catabolic and anabolic metabolism and the major acetyl donor in cells (Fig. 1) (4). In this manner, acetyl-CoA and numerous other endogenous metabolites exert known regulatory roles on histone- and DNA-modifying enzymes.

Here we will refer to endogenous metabolites as those generated by the mammalian host (e.g. mouse and human). A major source of metabolic diversity is encoded in the genomes of microbes that colonize the gut of mammals. These communities have co-evolved with their hosts (5) and interact with dietary components and host-derived molecules to produce a myriad of metabolites that are measurable in host circulation and tissues and can modulate physiology and behavior (6, 7). For example, butyrate, a major product of gut microbial fermentation of undigested complex carbohydrates, has been known as a histone deacetylase inhibitor since the 1970s (8). The relationship between SCFAs and a number of other microbial metabolites with host chromatin is detailed in Table 1. In light of these relationships, the gut microbiota may be a key regulator of host metabolob-epigenetic events. The gut microbiota has also been implicated in a number of host metabolic and immunological etiologies (9, 10).

Gut microbial communities and their hosts communicate via chemical signals in the form of small molecule metabolites and signaling molecules like LPS and peptides (7). Given its sensitivity to metabolite availability, a significant portion of this chemical communication may take place at the level of the host.

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Histone acetylation is generally associated with open chromatin states and active transcription. Acetylation leads to charge neutralization of lysine residues, affecting electrostatic interactions between DNA and residues within histone octamers. Acetylated residues also serve as binding sites for other factors that can play a role in transcriptional activation, including histone-modifying and chromatin-remodeling enzymes, as well as transcription factors (12). Histone methylation is associated with both transcriptional activation and silencing, depending on both the location and the degree of methylation of a particular lysine residue on the histone tail. For example, trimethylation at histone H3 lysine 4 (H3K4me3) is found at active or poised promoters (13), whereas H3K4me1 is typically associated with enhancers (14). In contrast, H3K27me3 is located in areas of closed chromatin or transcriptionally silenced genes (15). Similar to histone acetylation, methylated residues also serve as binding sites for a number of regulatory factors (16). Thus, histone PTMs create what has been termed the “histone code,” which consists of combinatorial histone PTM states that serve as both a signal integration platform for diverse environmental signals and landing platform for other effectors.

Histone-modifying enzymes are sensitive to levels of endogenous small molecule metabolites, with some serving as co-substrates while others act as inhibitors. The $K_m$ or $K_i$ values of many of these enzymes for their substrates or inhibitors, respectively, are often higher than measured or calculated levels of key metabolites, opening the possibility that fluctuations in these metabolites may regulate enzyme activities. The relationship between metabolism and histone-modifying enzymes and their kinetic parameters has been reviewed thoroughly in Ref. 4. Here we focus on histone acetylation and methylation, as the most common and well-studied histone PTMs in relation to metabolism. The interplay between endogenous metabolites and histone and DNA modification is depicted in Figs. 1–3.

Histone acetylation is the result of dynamic balance between the activities of HATs and histone deacetylases (HDACs). HATs catalyze the transfer of an acetyl group from acetyl-CoA onto the ε-amino group of lysine residues, releasing coenzyme A (CoA). Notably, coenzyme A acts as a competitive inhibitor of HATs. Acetyl-CoA also serves as a hub for central carbon metabolism with roles in catabolic, anabolic, and energy-producing pathways. Given its dual role as a necessary substrate for HAT enzymes and a central metabolite, acetyl-CoA is a rheostat that communicates cellular metabolic states to chromatin, ultimately regulating transcriptional programming. Cellular concentrations of acetyl-CoA are reported to be 2–20 μM, which is above the $K_m$ value for the HATs GCN5 and P/CAF but near the $K_m$ value of p300 (4).

The subcellular compartmentalization of metabolic reactions is important to consider in the context of metabolite-driven regulation of histone PTMs. Acetyl-CoA is produced by a number of cytosolic and mitochondrial reactions. It can be made directly from acetate by acetyl-CoA synthetase 1 and 2 (AceCS1 and -2) in the cytosol and mitochondria, respectively. In mitochondria, acetyl-CoA is also produced via β-oxidation of fatty acids and oxidative decarboxylation of pyruvate by the pyruvate dehydrogenase complex (PDC). Mitochondrial...
acetyl-CoA condenses with oxaloacetate (OAA) to form citrate, which can be shuttled into the cytosol and converted back into acetyl-CoA and OAA by ATP citrate lyase (ACLY). ACLY has been demonstrated to be essential for histone acetylation in response to glucose in mammalian cells; however, supplementation with 1–5 mM acetate partially rescued histone acetylation in the setting of ACLY knockdown (17). Interestingly, both ACLY and PDC have been reported to localize to the nucleus in mammalian cells in response to growth stimuli and in concordance with increased histone acetylation and acetyl-CoA pools (demonstrated for PDC only) (17, 18). AceCS1 has also been demonstrated to be present in the nucleus (17), although its role

| Metabolite Class | Members | Source | Direct Microbial metabolite:epigenetic effects = putative or demonstrated? | Model System | Effects |
|-----------------|---------|--------|-------------------------------------------------|--------------|---------|
| SCFAs | Lactate (C3) | putative | in-vitro, mammalian cell culture | Weak HDAC inhibition (76) |
| | Acetate (C2) | putative | in-vitro, mammalian cell culture | Increases histone acetylation (17) |
| | Propionate (C3) | putative | in-vitro, mammalian cell culture; intestinal organoids | Weak-modest HDAC inhibition in-vitro (< butyrate) (77); Increase histone acetylation in HT-29 cells (78); modest increase in expression of HDAC3 and HDAC5 in gut organoids (79) |
| | Butyrate (C4) | demonstrated | in-vitro, mammalian cell culture, mouse, human | HDAC inhibition (8, 77), HAT activation, protection from colorectal cancer (61, 70); protection from high fat diet induced metabolic syndrome + associated with decreased HDAC activity (62); Increase histone acetylation in HT-29 cells (78); modest increase in expression of HDAC3 and HDAC5 in gut organoids (79) |
| | General (acetate, propionate, and butyrate) | demonstrated | mouse | Colonization affects host histone PTM states in multiple host tissues; SCFA supplementation of GF mice is sufficient to partially phenocopy histone methylation and acetylation patterns observed in colonized mice (71) |
| | Valerate (C5) | putative | Mammalian cell culture | Increase histone acetylation in HT-29 cells (78) |
| | Caproate (C6) | -- | -- | Does not induce histone H4 hyperacetylation in HT-29 cells (78) |
| Branched SCFAs (BCFAs) | Isobutyrate | putative | in-vitro, mammalian cell culture | Increase histone acetylation (78); Increase histone H3 acetylation in EBV-infected HH514-16 cells (80) |
| | Isovalerate | putative | mammalian cell culture | Increase histone H3 acetylation in EBV-infected HH514-16 cells (80) |
Table 1—continued

| Metabolite Class | Members | Source | Direct Microbial metabolite:epigenetic effects = putative or demonstrated? | Model System | Effects |
|------------------|---------|--------|-------------------------------------------------|---------------|---------|
| **Organic Acids (other)** | Succinate | Fermentation of microbial accessible carbohydrate | putative | *in-vitro*, mouse model, yeast, subset of human cancers | Succinate and fumarate (a structural analog) are both known inhibitors of JmjC histone demethylases and TET family of DNA demethylases (31); hypermethylation in human tumors with succinate dehydrogenase mutation due to succinate accumulation (81) |
| B2 (riboflavin) | putative | -- | Precursor for FAD, a necessary cofactor for MTHFR in the folate cycle, important for SAM availability for HMTs and DNMTs (82) |
| B3 (niacin) | putative | *in-vitro*, yeast, and mammalian cell culture | Inhibitor of Class III HDACs (nicotinamide) (25) |
| B5 (pantothenate) | putative | -- | Required for biosynthesis of coenzyme A (CoA), which is necessary for production of acetyl-coA |
| B6 (pyridoxine) | Diet and from de-novo synthesis by microbiota | putative | Necessary cofactor in the folate cycle, important for SAM availability for HMTs and DNMTs |
| B9 (folate) | putative | mammalian cell culture, rodent, human | Colonic folate production by microbiota ≥ dietary intake (83); *Bifidobacterium* species demonstrated to produce folate in humans and rats (84, 85); folate is absorbed across colon (83); folate promotes availability of SAM for HMTs and DNMTs (4) |
| B12 (cobalamin) | putative | -- | Necessary cofactor for one-carbon metabolism, important for SAM availability for HMTs and DNMTs (82) |
| **Misc. Dietary Nutrients** | Choline | Diet | putative | In-vitro, in-silico, mouse model | Key dietary contributor to one-carbon metabolism and SAM availability for HMTs and DNMTs (82); metabolized by gut microbiota, limiting choline availability to the host, to produce TMA, which is absorbed by the host and further converted to |
### Table 1—continued

| Metabolite Class         | Members                  | Source                              | Direct Microbial metabolite:epigenetic effects = putative or demonstrated? | Model System                        | Effects                                                                                                                                 |
|-------------------------|--------------------------|-------------------------------------|---------------------------------------------------------------------------|-------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|
|                         |                          |                                     |                                                                           |                                     | trimethylamine-N-oxide (TMAO) in the liver (86, 87); choline deprivation during key developmental periods alters DNA methylation (82)           |
|                         |                          |                                     |                                                                           |                                     | See choline above; contributes to one-carbon metabolism and SAM availability for HMTs and DNMTs (82); dietary betaine modulation results in altered DNA methylation (88) |
| Betaine                 | Diet                     | putative                            |                                                                           | mammalian cell culture              | omega-3 PUFAs downregulated the methyltransferase EZH2, resulting in decreased H3K27me3 and attenuation of cancer phenotype (89); gut microbiota metabolize PUFAs to form conjugated linoleic and linolenic acids (90) |
| Polyunsaturated fatty acids (PUFAs) | Diet                     | putative                            |                                                                           | mammalian cell culture              | UDCA-treated cells are hypoacetylated on histones H2A, H3, and H4 and UDCA induces expression of HDAC6 (91); bile acid pool size and composition is affected by gut microbiota (72) |
| **Bile Acids**          | Ursodeoxycholic acid (UDCA) | Host-microbe cometabolism           | putative                                                                  | mammalian cell culture              | Gut microbiota metabolize ellagitannins to urolithins, which undergo enterohepatic circulation (92); reduce HAT activity, but not HDAC activity, in TNF-stimulated THP-1 cells (93) |
|                         | Ellagitannins (ellagic acid) |                                     | putative                                                                  | *in-vitro, cell culture*            |                                                                                                                                            |
|                         | ECGC (epigallocatechin-3-gallate) and theaflavins | Dietary: select fruits, vegetables, nuts, and teas; undergo extensive chemical transformations by microbiota in colon; undergo enterohepatic circulation | putative                                                                  | *in-vitro, cell culture*            | Inhibition of DNMT3a by ECGC and theaflavins *in vitro* (94); EGCG inhibits DNMT and HDAC activity, decreases expression of DNMTs, causes global hypomethylation, alters histone methylation and acetylation in a dose dependent manner (95) |
|                         | Phenolic acids (cinnamic and benzoic acids) |                                     | putative                                                                  | *in-vitro*                          | weak histone deacetylase inhibition (< propionate, << butyrate) (77)                                                                 |
|                         | Flavonoids (e.g., catechin, quercetin) |                                     | putative                                                                  | *in-vitro, cell culture*            | Inhibit DNMT1 *in-vitro* and cause DNA hypomethylation in cultured cell lines (96)                                                  |
in histone acetylation in cultured mammalian cells appears to be secondary to ACLY. The fact that these enzymes can translocate to the nucleus suggests that, beyond subcellular compartmentalization, metabolite availability may also be regulated at the level of subnuclear microenvironments and perhaps direct channeling from one enzyme to the other. Additional evidence for this link between cellular metabolism and histone acetylation comes from studies in yeast, where yeast metabolic cycles are associated with histone acetylation and regulation of growth-related genes (19).

Mammalian HDACs are organized into four classes, depending on their homology to yeast orthologues and their factor dependence: class I, IIa, IIb, and IV are all zinc-dependent deacetylases and are generally inhibited by hydroxamic acid inhibitors, including TSA (trichostatin A) and SAHA (suberoylanilide hydroxamic acid, Vorinostat), which chelate the active-site zinc. These metal-dependent HDACs catalyze the hydrolysis of acetyl groups from acetyl-lysine residues, producing acetate and a deacetylated substrate. The class III HDACs, also known as sirtuins, are structurally distinct from other classes of HDAC. Sirtuins require NAD$^+$ as a necessary co-substrate and produce nicotinamide, O-acetyl-ADP-ribose, and the deacetylated substrate. There are seven mammalian sirtuins (Sirt1–7), which share a conserved NAD$^+$-binding site and catalytic domain but diverge in their biological roles due to differences in subcellular localization, tissue expression, and substrate specificity (20). Of the sirtuins, Sirt1 and Sirt6 are localized to the nucleus, whereas Sirt7 is found in the nucleolus, and Sirt2–5 are either mitochondrial or cytosolic. The discovery that yeast orthologue Sir2 (silent information regulator 2) is regulated by NAD$^+$ availability was one of the first reports of a small molecule metabolite regulating chromatin modifications (21). More recently, the histone H3 Lys-9 and Lys-56 deacetylase Sirt6, which has inherently low deacetylase activity in vitro, was reported to be activated by long-chain free fatty acids (20); however, whether these long-chain free fatty acids have a role in vivo remains to be determined.

NAD$^+$/NADH is one of the most important redox enzymes found in living cells. It plays a role in both catabolic and oxidative pathways, including glycolysis, the TCA cycle, and oxidation of fatty acids. In addition to sirtuins, two other nuclear enzymes may be significant consumers of NAD$^+$: poly(ADP-ribose) polymerase (PARP) and CD38. PARP-1 is activated in response to genotoxic stress and is known to induce a caspase-independent form of cell death termed “parthanatos” (22), which was thought to be caused by excessive consumption of NAD$^+$ and bioenergetic collapse. However, it has recently been shown that the resulting bioenergetic collapse is not dependent upon NAD$^+$ depletion, but rather is due to inhibition of hexokinase and subsequent glycolytic defects by poly(ADP-ribose), a product of the PARP-1 reaction (23). Although precise measurement of subcellular NAD$^+$ has been limited by technical challenges and the fact that the majority of NAD$^+$ is protein-bound, nuclear NAD$^+$ has been estimated to be $\sim 70$–$109$ $\mu$M, which is approximately at or below the $K_m$ value of yeast Sir2 ($\sim 100$ $\mu$M) and mammalian Sirt1 ($\sim 150$–$170$ $\mu$M) but not Sirt6 ($K_d = 27$ $\mu$M), which can bind NAD$^+$ in the absence of a peptide substrate, suggesting it exists in a poised state (4, 24). NAD$^+$ and its role in metabolic signaling have been thoroughly reviewed in Ref. 25.

Histone methylation is balanced by the activities of histone methyltransferases (HMTs) and histone demethylases. Regulation of histone methylation by central carbon and one-carbon metabolites is depicted in Figs. 2 and 3, respectively. Histone methyltransferases fall into one of three major families of enzymes, all of which catalyze the addition of a methyl group to the ε-amino group of lysine residues or the guanidinyl group of arginine residues: 1) SET domain-containing enzymes; 2) DOT1-like enzymes, which methylate lysine residues; and 3) the protein arginine N-methyltransferase family of enzymes that methylate arginines. Although these enzymes have different target specificities, mechanisms, and kinetic properties, all known histone methyltransferases use S-adenosylmethionine (SAM, also known as AdoMet) as a donor and release...
S-adenosylhomocysteine (SAH, also known as AdoHcy) as a product. Methylation has also been reported to occur on histidine, cysteine, aspartate, and glutamate residues, although these modifications are much more rare and the biological significance remains to be determined. Methylation of lysine residues is the predominant form of histone methylation and exists in mono-, di-, and tri-methyl forms. As such, we will limit our focus here to methylation of lysine residues and its regulation by small molecule metabolites. Histone methylation and biological roles have been thoroughly reviewed in Ref. 16.

HMTs are regulated by the availability of the methyl donor SAM. This essential co-substrate is synthesized via the one-carbon cycle (also known as the methionine cycle), which utilizes methyl groups derived from dietary folate in the folate cycle (Fig. 3). These two cycles intersect at the vitamin B12-dependent enzyme 5-methyltetrahydrofolate homocysteine methyltransferase (methionine synthase, MTR), where a one-carbon unit from the folate cycle is used to convert homocysteine to methionine. Methionine adenosyltransferase then catalyzes the formation of SAM from methionine and ATP. SAM concentrations are reported to be 10–100 μM. This is slightly above the measured Km values for both SET domain-containing and non-SET domain-containing HMTs; however, SAH also competitively inhibits SAM binding to HMTs. SAH concentrations are reported to be 0.1–20 μM.

Betaine, B-vitamins, methionine, and folate (27) are dietary contributors for these pathways, including serine, glycine, choline, and SAH. The relationship between folate and one-carbon metabolism and HMT and DNMT activity is further detailed in Fig. 3. Histone demethylases are regulated by central carbon metabolites and carry out a redox reaction to remove methyl groups from histone lysine residues, producing formaldehyde. The LSD family of demethylases require FAD as an electron acceptor, producing FADH₂, whereas the JmjC family uses α-KG as a co-substrate and requires both oxygen and iron. The TCA cycle intermediates succinate and fumarate inhibit JmjC family demethylases.

In mammalian cells, SAM concentrations are reported to be 10–100 μM. This is slightly above the measured Km values for both SET domain-containing and non-SET domain-containing HMTs; however, SAH also competitively inhibits SAM binding to HMTs. SAH concentrations are reported to be 0.1–20 μM.
which is within the $K_i$ value of both SET and non-SET domain-containing HMTs (4). Thus, it is possible that the ratio of SAM/SAH, which differs by cell type and environmental conditions, is a biologically relevant measure of enzyme activity.

Histone demethylases are also closely coupled to cellular metabolic state (Fig. 2). There are two main classes: the FAD-dependent lysine-specific demethylase (LSD) family demethylases and the $\alpha$-ketoglutarate ($\alpha$-KG)-dependent JmjC family demethylases. Using different oxidizing agents, both families carry out a redox reaction to remove the methyl group from histone lysine residues, producing formaldehyde. The LSD family of demethylases uses FAD as an electron acceptor, generating FADH$_2$ (29), whereas the iron-dependent JmjC family uses oxygen and $\alpha$-KG and generates CO$_2$ and succinate (30). Interestingly, mutation of fumarate hydratase and succinate dehydrogenase in a subset of human cancers leads to accumulation of fumarate and succinate, respectively, both of which have been demonstrated to inhibit the $\alpha$-KG-dependent JmjC family of histone demethylases, causes aberrant histone and DNA methylation (31, 32).

Iron availability has also recently been demonstrated to affect histone PTM states in mouse myoblast cells, wherein pharmacological iron chelation resulted in reversible increases in histone methylation at JmjC target sites (33). Finally, hypoxia, which is a hallmark of a number of inflammatory conditions and tumor microenvironments, has also been shown to affect histone methylation via inhibition of oxygen-dependent JmjC family demethylases (34, 35). Thus, in a manner similar to acetyl-CoA, these key central carbon metabolites serve as TCA cycle intermediates ($\alpha$-KG, succinate, fumarate, and FAD), play roles in other oxidative processes such as $\beta$-oxidation of fatty acids and oxidative phosphorylation (FAD) and amino acid metabolism ($\alpha$-KG), and signal cellular metabolic status to chromatin.

**Cross-talk between DNA methylation, histone modification, and metabolites**

DNA methylation occurs mainly at CpG residues in the genome, and $\sim$60–80% of the mammalian genome is methylated; however, in regions of active chromatin only $\sim$10% of CG sites are methylated (36, 37). DNA methylation is associated with repressed transcription and closed chromatin. Some cross-talk between histone modification and DNA methylation has been established, particularly between H3K9 methylation and DNA methylation in the fungi *Neurospora crassa*, although it remains unclear which is the causative event (38, 39). Regardless, DNMTs have the same requirements for the methyl donor SAM as HMTs and thus are also regulated by nutrient availability and cellular metabolic state, as shown in Fig. 3. For example, both *in utero* and early life adversity have been shown to affect DNA methylation, in some cases affecting multiple generations (2, 3). Recently, fumarate has also been shown to drive the epithelial to mesenchymal transition, a key step in tumor invasion and metastasis, by inhibiting TET (ten eleven translocation)-mediated demethylation of an anti-metastatic miRNA cluster (32). Thus, similar to histone methylation, both one-carbon and central metabolites can chemically signal to DNA methylation machinery.

**Histone PTM states: Regulation by gut microbial metabolites**

The gut microbiota produces a large number and variety of bioactive metabolites (6, 7), including both demonstrated and putative regulators of host chromatin as follows: SCFAs, vitamins, bile acids, and compounds derived from metabolism of dietary components, including polyphenols, isothiocyanates, and choline (Table 1). Gut microbial community composition affects metabolic outcomes; for example, the number of genes within a gut microbiome (richness) correlates with metabolic biomarkers (40). Furthermore, dietary intervention has been shown to improve low gene richness and subsequent clinical phenotypes (41). In a small human cohort study, consumption of either an entirely animal- or plant-based diet resulted in alterations in microbial diversity within 1 day of consumption of the altered diet, and consumption of the animal-based diet increased the abundance and activity of *Bilophila wadsworthia* (42), which has been associated with inflammatory bowel disease (43). Dietary additives common to Westernized human diets cause gut dysbiosis and contribute to metabolic syndrome (44) and gut inflammation (45). Although host genetics have been shown to play a role in shaping gut microbial community composition and metabolism, the effects of diet and environment have been shown to exert broader effects (46, 47).

Although the gut microbiota is necessary for proper immune system and brain development (48, 49), several studies have shown that it contributes to a number of etiologies, including metabolic syndrome and diabetes mellitus (50, 51), obesity and adiposity (52, 53), cardiovascular disease (54, 55), non-alcoholic fatty liver disease (56), inflammatory bowel disease (57), and colon cancer (58). Furthermore, changes in microbiota composition caused by antibiotic exposure early in life affect the gut microbiota and elicit long-lasting effects on host metabolic outcomes (59, 60). Notably, the gut microbiota is also associated with therapeutic effects (61, 62).

There are a number of interesting and putative connections between microbial-host metabolic axes and chromatin regulatory events; however, most of these studies have provided only indirect evidence or used cell culture-based models rather than whole organisms. For example, it is well established that early life adversity affects DNA methylation (63). This is a critical time in life when the microbiome is assembled (64, 65). Interestingly, adverse events during this key developmental period (either *in utero* or during early life) have been shown to impact both chromatin and the gut microbiota; however, with the exception of microbial production of butyrate in the setting of colon cancer (61), these two have not yet been directly linked. Furthermore, natural seasonal variation in nutrient availability has been linked to alterations in both chromatin states and the microbiota (2, 66). Similar effects have also been separately reported for high fat diet feeding on chromatin and the microbiota (67, 68). Even more intriguingly, Sonnenburg *et al.* (69) recently showed that diet-microbiota interactions may reprogram transgenerational susceptibility to metabolic disease. Although consumption of a diet low in microbial accessible carbohydrates (MACs) induces largely reversible effects on the gut microbiota within a single generation, continued feeding of
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a low MACs diet results in loss of microbial taxa that are at increased risk for extinction with each subsequent generation. Thus, key links exist between microbiota-dependent transgenerational effects and potential metabolic effects associated with consumption of a typical Westernized diet (which is low in MACs). Although these effects were not linked to chromatin states, this study presents the intriguing possibility that perhaps transgenerational inheritance in response to nutrient availability is mediated via gut microbiota-host epigenetic responses.

A large number of microbial metabolites have been measured in host circulation and other tissue compartments largely via NMR and mass spectrometry (6, 7, 67). These studies highlight the extensive co-metabolism that occurs between the gut microbiota and host. Table 1 details demonstrated and putative interactions between host epigenetic machinery, gut microbial metabolites, and host-gut microbial co-metabolites. Of note, the SCFAs acetate, propionate, and butyrate are the only examples for which a direct link between microbial metabolite production and host epigenetic programming has been made in a whole organism. Donohoe et al. (61, 70) demonstrate a key link between gut microbial fermentation of dietary fiber and both normal maintenance of healthy colonic epithelium and attenuation of colon cancer via butyrate-mediated effects on histone acetylation and gene expression. Recently, we have also demonstrated that the gut microbiota affects global histone methylation and acetylation and that these effects can be partially mimicked in GF mice that are supplemented with a mixture of p-cresol and branched SCFAs (71). To our knowledge, all other relevant studies have either used purely in vitro methods or involved treatment of cell culture-based models with known microbial metabolites. Thus, there remains enormous potential for discovery, which links commonly available foodstuffs to epigenetic programming in health and disease.

In addition to butyrate, other organic acids (C1, C2, C3, and C5 and branched SCFAs) have been demonstrated to increase histone acetylation, inhibit HDACs, or increase expression of HDACs in cell culture models (Table 1). The organic acid succinate has also been demonstrated to inhibit both histone and DNA demethylases (Table 1). Another major group of co-metabolites are B-vitamins, which are both derived from diet and synthesized de novo by gut bacteria. Vitamins B2, B6, B12, and all play roles in SAM availability and thus may affect histone and DNA methylation, whereas vitamins B3 and B9 may affect histone acetylation via sirtuin inhibition or HAT activation, respectively (Table 1). Other dietary nutrients, including choline, betaine, and polyunsaturated fatty acids (PUFAs), may play roles in histone methylation (Table 1).

Bile acids are regulators of gut microbial community composition and are also regulated by the gut microbiota via microbial production of secondary bile acids that mediate both bile acid pool size and composition (72). The human secondary bile acid ursodeoxycholic acid (a primary bile acid in mice) also induces expression of HDAC6 and decreases global histone acetylation in cultured cells (Table 1). Finally, two classes of phytonutrients that are metabolized by the gut microbiota to bioactive compounds have putative roles in host epigenetic regulation. Both polyphenol metabolites and glucosinolates are derived from plants (select fruits, vegetables, nuts, and teas) and are metabolized by the gut microbiota to form bioactive compounds that may regulate host chromatin at the level of methylation and acetylation of histones as well as DNA methylation (Table 1).

Although gut microbial derivatives of dietary isothiocyanates and polyphenols are potential regulators of host epigenetic machinery, their bioavailability is somewhat limited, and thus future studies will need to determine the relevance of these metabolites in this setting. Interestingly, organic acid production in the distal gut is associated with a decrease in pH (73). This is particularly intriguing within the context of work by McBriar et al. (74), wherein histones are globally deacetylated as extracellular pH decreases and hydrolyzed histone acetyl anions are exported with protons as a means to regulate intracellular pH. This suggests that organic acid production in the colon may promote decreased histone acetylation; however, this is at odds with reports of SCFA-driven increases in histone acetylation. Perhaps organic acid-driven effects on pH and on HDACs/HATs are cell type- and tissue-specific.

Conclusions

Much of the extensive chemical communication that occurs between the gut microbiota and host may occur through chromatin-mediated mechanisms. A number of microbial metabolites exert physiological effects via cellular signaling pathways and can even exert effects via multissite signaling, as demonstrated for glucose homeostasis via gut-brain neural circuits (75). These signaling effects need not be mutually exclusive from chromatin effects, however, emphasizing the importance of elucidating chromatin effects in response to the multitude of gut microbial metabolites. Furthermore, as both proteomic and metabolomic methodologies continue to improve, the identification of novel metabolite-epigenome interactions will drive further exploration of this exciting interaction between the host and its microbial symbionts, undoubtedly yielding key insights into how the microbiota modulates the health of the host.

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