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Gut microbiota–generated metabolites in animal health and disease

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Gut microbiota is found in virtually any metazoan, from invertebrates to vertebrates. It has long been believed that gut microbiota, more specifically, the activity of the microbiome and its metabolic products, directly influence a variety of aspects in metazoan physiology. However, the exact molecular relationship among microbe-derived gut metabolites, host signaling pathways, and host physiology remains to be elucidated. Here we review recent discoveries regarding the molecular links between gut metabolites and host physiology in different invertebrate and vertebrate animal models. We describe the different roles of gut microbiome activity and their metabolites in regulating distinct host physiology and the molecular mechanisms by which gut metabolites cause physiological homeostasis via regulating specific host signaling pathways. Future studies in this direction using different animal models will provide the key concepts to understanding the evolutionarily conserved chemical dialogues between gut microbiota and metazoan cells and also human diseases associated with gut microbiota and metabolites.

Gut microbiota and its metabolites have a pivotal role in the maintenance of homeostasis of metazoan physiology. Mucosal surfaces covered by a layer of gut epithelia represent the most critical interface between the organism and its environment. It is now commonly known that gut cell homeostasis influences diverse elements of host physiology, including development, metabolism and immunity. The relationship between gut and host health and disease is well documented, although detailed molecular mechanisms remain obscure. Over a century ago, the Russian scientist Elie Metchnikoff (1845–1916) conceptualized the role of the gut on host physiology and pathology[1]. He suggested that gut homeostasis is key to many components of host physiology, including longevity. He also proposed that gut bacteria are essential modulators influencing homeostasis and that deregulation of gut homeostasis by certain bacteria leads to a diseased state owing to poisoning of the body from bacterial byproducts. Unfortunately, Metchnikoff's hypothesis of relationship between gut metabolites and host health remained dormant for nearly a century[1]; his hypothesis has only recently reemerged as one of the most active areas of investigation in modern biology and medicine. Gnotobiotic animal models with genetically tractable 'microbe-host' are now enabling us to see a clearer picture of host genome interactions with the microbiome or metabolites. In this review, we will discuss the most up-to-date discoveries on the evolutionary conserved chemical dialogs between gut microbiomes and metabolites and different metazoans and also on human diseases associated with gut microbiota and metabolites.

Gut metabolites and their roles in invertebrates

Classical invertebrate animal models for developmental biology, such as *Caenorhabditis elegans* and *Drosophila melanogaster*, are now successfully established in the field of gut-microbe interactions[2–6]. The simplicity of the gut microbiota or microbiome, genetic tractability of host animals and easy generation of gnotobiotic animals (by simply bleaching eggs) has enabled the quick and decisive advancement of our understanding of gut-microbiota interactions compared to vertebrate models. For example, *C. elegans* and *Drosophila* harbor extremely simple gut microbiotas under laboratory conditions (for example, 1 bacterial species in *C. elegans* and 5–20 species in *Drosophila*), and all of these bacteria can be cultured *in vitro*, enabling the genetic manipulation of microbiomes[2,3,5,7]. Given that these animals have short life spans and hundreds of individuals can be easily used for different experiments under rigorously controlled diets and microbial conditions, *C. elegans* and *Drosophila* became the models of choice for the genetic analysis of complex phenotypes, such as animal aging and development[8]. Not surprisingly, these animal models have been used to show the biological significance of interkingdom interactions by showing the unexpected roles of the microbiome in metazoan longevity, dysbiosis and development.

Gut metabolites and host longevity. It has long been observed that metazoans live in close relationship with microorganisms. The soil-dwelling worm *C. elegans* eats microorganisms as a part of its diet. Although they consume *E. coli* OP50 strain under laboratory-rearing conditions, it was shown that they prefer to consume soil bacteria, such as *Bacillus mycoides* and *Bacillus soli*[9]. As such nutrition involves metabolically active microorganisms, it can generate different metabolites. Previously, it has been shown that the metabolically active state of microorganisms is important in conferring benefits to host growth and longevity[10]. Furthermore, altered *E. coli* metabolism, such as reduction of the bacterial respiration rate, can extend worm longevity[11]. Interestingly, feeding worms with soil bacteria, as opposed to *E. coli* OP50, was found to extend worm life span[9]. All of these observations indicate that some metabolites generated by specific microbiome activity directly affect host growth and longevity. Recently, it has been shown that bacterial nitric oxide (NO) produced by bacterial NO synthase (NOS) activity enhances host longevity and stress resistance in *C. elegans*[2] (**Fig. 1**). *C. elegans*, unlike many other metazoans, is unable to produce NO in a NOS-dependent manner; thus, it relies solely on bacterial NO supply. Further genetic studies elegantly demonstrated that bacterial NO modulates DAF-16 (a homolog of FOXO) and HSF-1 (a master transcription factor controlling the expression of heat shock proteins) that in turn induce aging-related genes capable of enhancing host longevity and the anti-stress response[2] (**Fig. 1**). Many intriguing questions remain to be answered. Especially, as many bacteria including human gut microbiota are capable of producing NO, it

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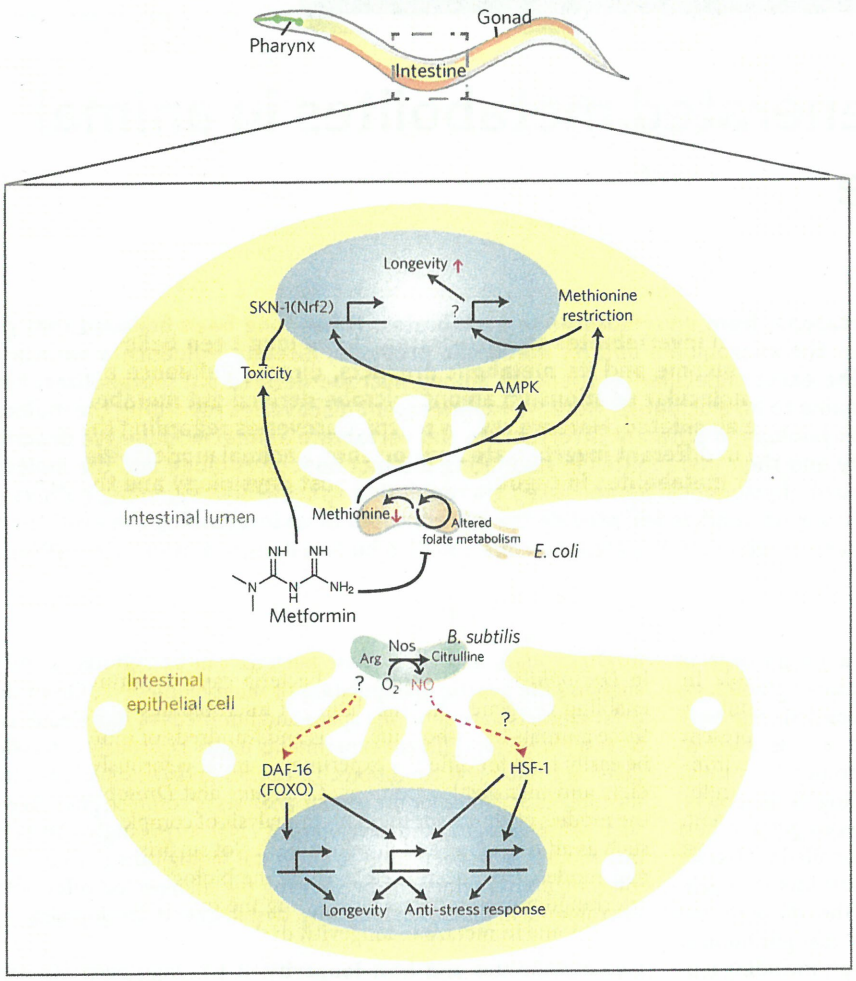

Figure 1 | Gut metabolites and metabolism and host longevity in *C. elegans*. Bacterially derived NO modulates the expression of genes involved in longevity and anti-stress responses via DAF-16 and HSF-1 activity. Metformin administration induces reduction of bacterial methionine production by disrupting the folate cycle. Reduced levels of methionine in the diet in turn induce the dietary restriction effect to promote expression of genes involved in longevity as well as genes involved in drug detoxification in an AMPK- and SKN1-dependent manner.

would be interesting to examine any health-promoting effects of bacterial NO in the human gut.

Given that bacterial metabolic activity can influence the host signaling pathway, researchers are now giving special attention to investigate whether environmental factors, such as xenobiotics or nutrition (that are capable of positively or negatively influencing host fitness), exert their effects by directly or indirectly modulating gut bacterial metabolism[12–16]. In particular, the role of bidirectional interactions between drugs and gut microbiomes on host physiology has been extensively discussed[15,16]. One remarkable observation has been made on the effect of metformin on host longevity. Metformin is a dimethyl-biguanide, a biguanide derivative, that is a most widely used anti-diabetic drug[17]. In the case of mammals, metformin administration has recently been found to alter the gut commensal community[18,19]. It has also been shown to enhance growth of the mucin-degrading bacterium, *Akkermansia* spp., which is accompanied by increased levels of mucin-producing goblet cells in the gut and Foxp3$^+$ regulatory T (T_{reg}) cells in adipose tissues[19]. Oral administration of *Akkermansia muciniphila* without metformin to diet-induced obese mice is sufficient to improve glucose tolerance along with reduced adipose tissue inflammation[19]. This observation indicates that metformin-induced dominance of a specific gut bacterium in mice mediates, at least in part, the anti-diabetic effect of metformin. In addition to its anti-diabetic effect, metformin is also known to induce life span in rodents and *C. elegans*[20,21]. Recently, it has been shown that that metformin alters bacterial folate and methionine metabolism, resulting in methionine restriction in *C. elegans*[3] (**Fig. 1**). The metformin-induced methionine restriction subsequently results in diet restriction, leading to a life span extension in *C. elegans* (**Fig. 1**). Methionine restriction in the diet is known to positively affect the longevity of other metazoans, from *Drosophila* to rodents[22,23], although the exact mechanism remains to be elucidated. However, addition of metformin to the diet of *Drosophila* did not enhance their life span[24]. Considering that metformin enhances host longevity by altering metabolism of gut microbiota, one possible explanation could be that metformin may be incapable of altering bacterial folate and methionine metabolism in the case of the *Drosophila* gut bacteria. As *Acetobacter* spp. and *Lactobacillus* spp. are two major gut commensal bacteria in *Drosophila* gut[25], it would be interesting to examine whether folate and methionine metabolism of these bacteria is influenced by metformin treatment. All of these findings illustrate unexpected roles of the gut microbiota and microbiome on animal longevity, providing a new concept of microbiome- and metabolite-based longevity medicine.

Gut metabolites and host dysbiosis. Gut epithelia are delicate barriers that are exposed to hostile bacterial environments. Maintaining a health-promoting bacterial community structure is of paramount importance for host fitness (the phenomenon is called gut-microbe symbiosis)[26]. Deregulation of bacterial community structure (for example, altered bacterial community members or overgrowth of certain bacteria) may lead to dysbiosis[27], resulting in disease phenotypes in the host. The sequential molecular events leading to dysbiosis are presently unclear. It was thought that symbiotic resident bacteria contribute in part to the maintenance of symbiosis by eliminating environmentally derived nonresident bacteria[28]. For example, gut bacteria prevent colonization of potentially harmful bacteria by competing for available nutrition or by releasing antimicrobial agents[28]. In addition to the effort made by symbiotic bacteria, host gut immunity is also known to participate to maintain gut-microbe symbiosis while suppressing dysbiosis.

In *Drosophila*, two major gut immune effectors operate to control gut bacteria; dual oxidase (DUOX)-dependent (DUOX is a member of the NADPH oxidases) microbicidal reactive oxygen species (ROS) and Relish, a homolog of p105-like NF-κB transcription factor–mediated antimicrobial peptide (AMP)[29,30]. It is known that the bacterially derived molecules, uracil and peptidoglycan, act as agonists for PLCβ-dependent DUOX activation and immune deficiency (IMD)-dependent Relish activation (where IMD is a homolog of the mammalian NF-κB pathway), respectively[29,30]. It should be noted that uracil is released from pathobionts (normally benign gut bacteria that are conditionally pathogenic when the commensal community is deregulated) or environmentally driven

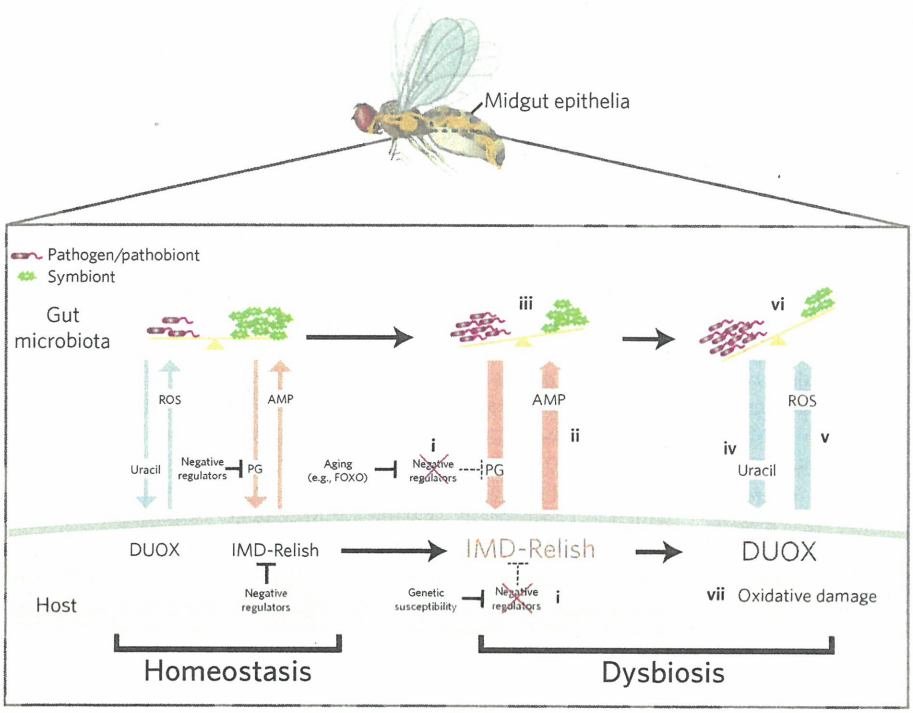

Figure 2 | A model for gut metabolites and dysbiosis in *Drosophila*. Two bacterially derived metabolites, peptidoglycan (PG) and uracil, induce adequate intensity of IMD-Relish and DUOX activation, respectively. IMD-Relish and DUOX activation induce production of two different microbicidal effectors, AMP and ROS, respectively, to control the gut bacteria, which is essential for gut-microbe homeostasis. The sequential events leading to dysbiosis are speculated on, on the basis of previous publications[6,25,31–34]: (i) the loss of negative regulations (either by genetic susceptibility to the IMD pathway due to the mutation of the negative regulators or by repression of negative regulators, such as PG-degrading enzyme through aging-dependent FOXO activation), (ii) AMP overproduction due to constitutive IMD pathway activation, (iii) modification of the commensal community, such as overgrowth of uracil-producing pathobionts due to the activity of AMPs, (iv) DUOX overactivation due to the high level of bacterially derived uracil, (v) excess production of DUOX-dependent ROS, (vi) further modification of commensal community due to the high ROS level, (vii) oxidative damages to host leading to host death.

pathogens but not from symbionts[31]. In contrast to this, peptidoglycan is released from most gut bacteria regardless of whether they are symbionts, pathogens or pathobionts[31]. Normal conventional gut environments (i.e., where symbionts are dominant) create a peptidoglycan-rich condition that could potentially induce constitutive IMD- and Relish-dependent AMP production. However, it has been found that different negative regulators suppress IMD- and Relish-dependent AMP production[25,32–34]. These negative regulations include peptidoglycan degradation by peptidoglycan-degrading enzymes, inhibition of the IMD signal pathway and suppression of AMP gene expression by transcriptional repression[25,32–34]. Mutant animals with a defect in one of these negative regulators showed classical features of intestinal dysbiosis[25,32–34], such as constitutive IMD pathway activation (high AMP production), modification of the gut commensal community, gut cell apoptosis and early host death. As the germ-free state of these mutant animals abolishes all of the above disease phenotypes, the gut commensal community is a causal element of intestinal dysbiosis. These observations also indicate that homeostasis of the IMD pathway is required for maintenance of a healthy gut commensal community and host fitness. Similarly, NF-κB–controlled AMPs are also known to regulate the composition of the colonizing microbiota in hydra[35].

It has long been observed that intestinal dysbiosis is associated with aging. It is known that, when worms and flies age, bacterial loads increase in the intestine, which negatively influences animal life span[6,36]. Recently, Jasper and collaborators[6] showed that aging is indeed a dysbiosis-inducing factor in the *Drosophila* intestine, providing an important link between aging and dysbiosis. They found that aged *Drosophila* showed enhanced FOXO activation (i.e., nuclear translocation) in the intestine, which in turn repressed a member of the peptidoglycan-degrading enzymes. This situation causes chronic IMD- and Relish-dependent AMP production, modification of the commensal community, stem cell hyperproliferation and epithelial dysplasia, resulting in host mortality[6].

But how does the constitutive IMD pathway activation lead to host mortality? Previously, chronic IMD pathway activation (or AMP overexpression) was sufficient to promote overproliferation of a pathobiont[25]. Recently, bacterially derived uracil molecules released from a pathobiont have been shown to act as agonists to induce chronic DUOX activation and excess ROS production, which is responsible for early host death[31]. Given that uracil feeding alone can induce DUOX activation accompanied by stem cell deregulation as well as severe oxidative stress to the host animal[31], one could speculate that the loss of the negative regulation of the IMD pathway observed in aged flies could induce uracil-rich gut environments by supporting the expansion of uracil-producing pathobionts and/or by initiating a uracil excretion mechanism of a pathobionts (**Fig. 2**). Future investigations into the identification of factors directly involved in the host pathology and their modes of action will be of central importance in the improved understanding of the relationship between bacterial metabolism and metabolites and intestinal dysbiosis.

Gut metabolites and host development. It has long been observed that germ-free animals exhibit abnormal organ development and small body size, suggesting a role of gut microbiome and metabolites on animal growth and organ homeostasis. These phenomena have been observed in different invertebrates, including *C. elegans*[10], *Drosophila*[7], hydra[37] and squid[38]. In addition to their roles in immune activation, bacterially derived immune agonists are known to be involved in organ development including hydra and squid[39]. In the case of *Drosophila*, two major commensal bacteria, *Acetobactor* and *Lactobacillus*, are known to have an essential role in the animal development, especially under poor nutritional conditions[7,40]. In the case of *Lactobacillus*, bacteria modulate the host TOR-dependent nutrition-sensing mechanism and ecdysone and insulin hormone signaling to promote animal growth[40]. As this bacterium is also a commensal bacterium in many other metazoans, including humans, the genetic analyses of *Lactobacillus* and *Drosophila* may be an ideal model system to provide new insight into evolutionarily conserved probiotic effects. It has been shown that *Acetobacter* showed multiple effects on hosts, such as a larval growth-promoting effect and effects on adult cell size and number, organ size, sugar and lipid levels, intestinal stem cell regulation and animal size[7]. Genetic analyses of *Acetobacter*

genome further showed that the bacterial pyrroloquinoline quinone (PQQ)-dependent oxidative respiratory chain in the periplasmic membrane is required for all of the aforementioned effects as it induces host insulin signaling activation[7]. It is presently unclear how PQQ-dependent metabolism influences insulin signaling, thereby regulating host physiology. As absence of the growth-promoting effect in *Acetobacter* lacking PQQ-dependent metabolism (*Acetobacter*-ΔPQQ) can be ameliorated by the addition of acetic acid (an intermediate metabolite of PQQ-dependent metabolism), acetic acid production is required to achieve a host growth-promoting effect[7]. However, acetic acid alone (without the presence of *Acetobacter*-ΔPQQ) is not sufficient to promote growth, suggesting that both PQQ-dependent acetic acid and PQQ-independent metabolisms and metabolites are most likely required for insulin signaling activation and full growth-promoting effects. All of these observations in *Drosophila* suggest that specific bacterial metabolism can directly or indirectly influence a host's master metabolic signaling, such as insulin signaling. Identification of bacterial metabolites and the molecular mechanism by which they induce host development and the metabolic signaling pathways will give essential clues for unraveling the mechanisms of co-evolution between prokaryotes and eukaryotic hosts.

Physiological roles of gut metabolites in vertebrates

Gut metabolites and host metabolism. Human microbiome analyses have revealed that the intestinal microbiota is a complex community consisting of more than 500 species, with *Firmicutes* and *Bacteroidetes* as dominant phyla. The total genes of gut microbiota outnumber our genes by more than 100-fold[41]. Many of these microbial genes are involved in main metabolic pathways such as carbon metabolism and amino acid synthesis. Bacterial composition in human stool is diverse even among healthy subjects. However, the genes related to the metabolic pathway are stable among individuals, regardless of variation in bacterial composition[42], suggesting that maintenance of the core microbiomes involved in carbon and amino acid metabolisms is one of the key aspects of gut commensal community. As a consequence, intestinal microbiota intimately influence the metabolism of their mammalian hosts and critically contribute to mammalian physiology (**Fig. 3**). The gut microbiota in mammals, including humans and mice, is known to be commensal, which in Latin means 'sharing a dining table'. Commensal bacteria mediate extraction, synthesis and absorption of a wide variety of metabolites[43]. These metabolites have been associated with the host's metabolic phenotype. Germ-free (GF) mice, which are reared without exposure to any live bacteria, ingest 30% more food compared

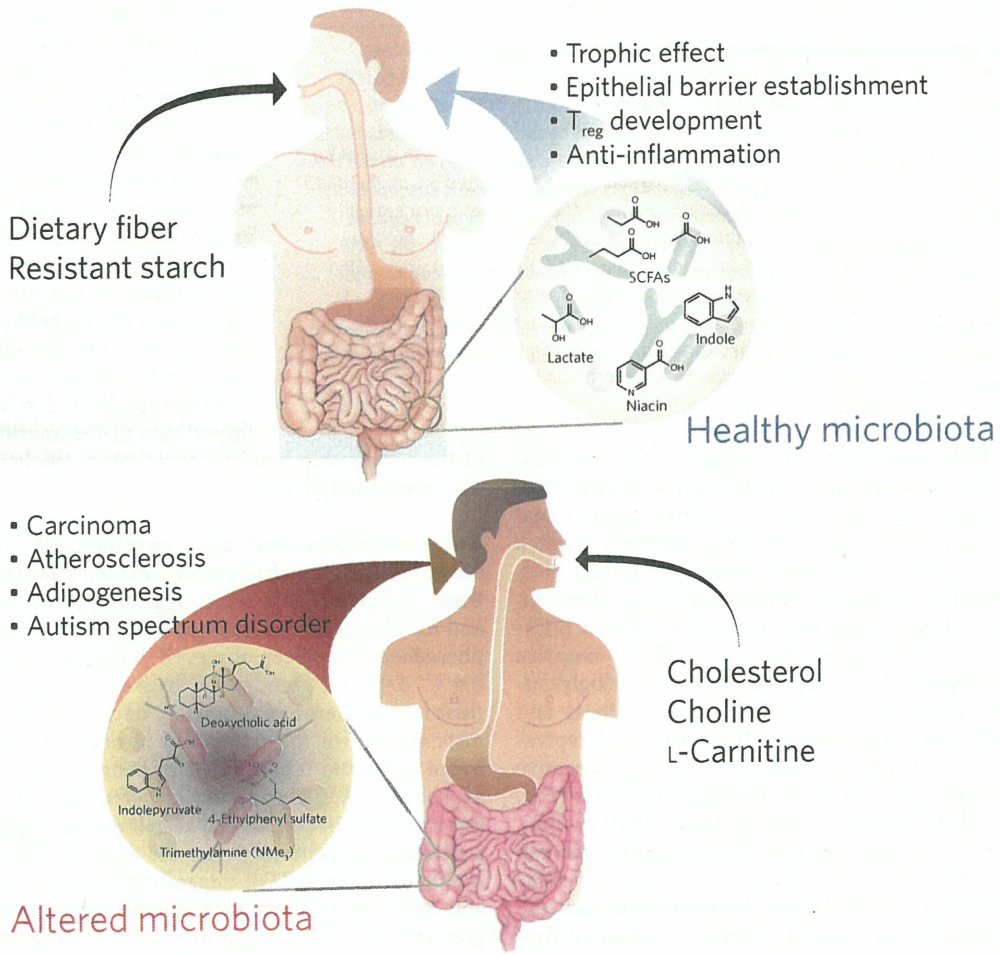

Figure 3 | Physiological and pathological roles of gut microbial metabolites. Gut microbiota are considered important environmental factors that affect the immune system, obesity, cardiovascular diseases and brain activity. Microbial metabolites are indispensable for the majority of the biological effects of gut microbiota. Under physiological conditions, soluble dietary fibers and resistant starch can be actively fermented by commensal microbiota in the large intestine. The fermentation products such as SCFAs have been appreciated for their beneficial effects on intestinal epithelium and the gut immune system. In contrast, HFD and inflammation disturb the microbial community, leading to dysbiosis (for example, the increased ratio of *Firmicutes* to *Bacteroidetes* at the phylum level and an increase in cluster XI of the genus *Clostridium*) and an altered metabolic profile in the intestinal lumen. Such dysbiosis-associated metabolites (or enhanced translocation of normal metabolites into the body) are implicated in systemic as well as gastrointestinal disorders.

with conventionally (CV) housed mice[44]. Nevertheless, GF mice are leaner, with approximately 40% less total body fat compared with CV mice. Conventionalization of GF mice by inoculation of microbiota from CV mice normalizes body fat mass within 2 weeks.

Soluble dietary fibers (for example, fructans, pectin, inulin and xylans) and resistant starch can be actively fermented by commensal microbiota in the cecum (rodents) and colon (humans), producing biologically active metabolites represented by short chain fatty acids (SCFAs), namely acetate, propionate and butyrate[45] (**Fig. 3**). Under physiological conditions, luminal concentrations of total SCFAs reach up to 80–130 mM in the human colon[46]. SCFAs serve as a major energy source for intestinal epithelial cells, in which up to 70% of energy intake is provided by gut microbiota. Colonic epithelial cells of GF mice under energy-deprived status are characterized by decreases in NADH/NAD^+ balance and ATP levels[47]. This eventually leads to phosphorylation of AMPK, a sensor of energy deprivation, and subsequent Cdkn1b/$p27^{kip1}$ activation to not only prevent apoptosis but also induce autophagy. The induction of autophagy in GF mice reflects nutrient starvation and may be important in maintaining cellular energy levels and in facilitating amino acid recycling for new protein synthesis through degrading unnecessary proteins and intracellular organelles[48]. Colonization of GF mice with the butyrate-producing bacteria *Butyrivibrio fibrisolvens*[49] rescued colonic epithelia from the energy starvation status and autophagy observed in GF colonic epithelium[47].

Production of SCFAs by commensal microbiota is implicated in adipogenesis in young mice treated with subtherapeutic doses of antibiotics[50]. This treatment increases adiposity without affecting body weight later in life. This finding is analogous with the practical experience that oral administration of low-dose antimicrobial agents is effective in promoting the growth of young farm animals. In antibiotic-treated mice, the ratio of *Firmicutes* to *Bacteroides* is augmented; the change is reminiscent of genetically obese *ob/ob* mice and obese people[51,52]. Exposure to antibiotics early in life results in enhanced production of SCFAs, increased secretion of incretin hormone GIP from K cells and upregulation of a group of hepatic genes related to lipogenesis and triglyceride synthesis in mice. These observations imply higher energy extraction from indigestible carbohydrates and enhanced adipogenesis in the liver. Thus, subtherapeutic antibiotic treatment in early life may perturb the gut microbial community, leading to long-term alterations of microbial composition. An imbalanced microbial community may affect gut microbial fermentation and host metabolism. In agreement with this view, an increase in energy harvest has been considered a predisposing factor for obesity[53]. Therefore, manipulation of gut microbiota may be a useful approach for regulating host metabolic phenotypes. Further studies are required to identify the specific microbial species that regulate energy harvest and to understand the molecular mechanism by which these bacteria affect host metabolism.

SCFAs serve as specific activators of Gpr41, Gpr43 and Gpr109a, which are encoded by *Ffar3*, *Ffar2* and *Niacr1*, respectively. Although Gpr41 and Gpr43 are activated by all three SCFAs, Gpr109a is only activated by butyrate[54]. Among these receptors, Gpr43 and Gpr109a are predominantly expressed in intestinal epithelial cells, adipocytes and myeloid cells (i.e., dendritic cells and granulocytes)[54–57]. In contrast, lymphocytes are devoid of both receptors. Gpr41 expression is more widely observed in immunological organs with little, if any, expression in peripheral organs[55]. Recently, several papers have linked Gpr43 with host-microbe metabolic interaction, although this remains controversial. It was initially reported that acetate and propionate promoted adipogenesis via Gpr43 in 3T3-L1 cells[58]. Furthermore, Gpr43-deficient mice fed a high-fat diet (HFD) displayed improved glucose tolerance and decreased body fat mass[59]. In contrast, other groups have reported that HFD-fed Gpr43-deficient mice exhibit obesity phenotypes characterized by an increase in white adipose in conjunction with progressive insulin tolerance and adipose tissue inflammation[60,61] and vice versa, i.e., the adipose tissue-specific transgene of *Gpr43* leads to a lean phenotype. Therefore, the expression of Gpr43 in adipose tissue is most likely responsible for lipid metabolism. The distinct phenotypes of Gpr43-deficient mice among different animal facilities may result from the different genetic backgrounds of the mice. An alternative interpretation may be that variation in intestinal microbiota communities, which is dependent on the environmental factors at each facility, may determine the metabolic phenotypes of host animals. It is noteworthy that Gpr43 deficiency leads to expansion of *Firmicutes* in gut microbiota and consequently raises fecal SCFA and plasma acetate levels, probably because of impaired gut immune homeostasis, for example, reduction of T_{reg} cells and uncontrolled neutrophil activation[62,63]. Because of the diverse biological functions of Gpr43, the metabolic characteristics of Gpr43 deficiency might be determined as the net results of altered lipid metabolism, microbial imbalance, the overproduction of SCFAs and impaired immune homeostasis. Future studies using adipose tissue or immune cell type–specific Gpr43 conditional knockout mice will provide a clue to the biological significance of Gpr43 in lipid metabolism.

It should be noted that gut microbiota also has an important role in absorption and catabolism of dietary phytochemicals, such as polyphenols. For examples, gut microbiota hydrolyzes glycosylated and/or polymeric polyphenols to yield aglycons that are further subjected to ring fission, leading to the production of phenolic acids and hydroxycinnamates[64]. The microbial transformations of the polyphenols lead to yield metabolites with altered bioavailabilities and bioactivities[65]. Similarly, the gut microbiota has a great impact on drug metabolism and drug-induced toxicity. There are many excellent reviews on the xenobiotic metabolism[15,16].

Gut metabolites shape the intestinal and systemic immune system. Compelling evidence has demonstrated that colonization of commensal bacteria critically contributes to the development of the mammalian immune system[66]. GF mice are defective in epithelial barrier functions[67], development of gut-associated lymphoid tissues (for example, isolated lymphoid follicles)[68], production of IgA[69] and generation of T_{reg} cells in the colon[70,71]. Remarkably, the effect of commensal microbiota is not only confined to the gut immune system but also extends to the systemic immune response[72]. Under GF conditions, the T_H1-T_H2 balance is skewed toward the T_H2 response and, correspondingly, serum IgE is elevated. Such an immunological phenotype of GF mice is normalized by exposure to a diverse microbiota during early life; however, exposure to a limited species of microbiota in early life or exposure to a diverse microbiota in adulthood (>12 weeks old) fails to reduce serum IgE levels[73]. Thus, exposure to diverse microbiota in early life is most likely required to shape IgE induction pathways, providing experimental evidence for the hygiene hypothesis[74].

Although the complete mechanisms by which gut microbiota regulates host immunity have yet to be elucidated, microbiota-derived products at least partly mediate the immunomodulatory effect. Such bioactive materials are produced by microbiota in both diet-dependent and independent manners[66]. The diet-independent products are mainly bacterial cell wall components, such as lipopolysaccharides and peptide glycans, both of which elicit innate immune response through the activation of Toll-like receptor 4 (TLR4)-dependent and nucleotide-binding oligomerization domain (NOD) receptor–dependent signaling pathways, respectively[28]. *Bacteroides fragilis* polysaccharide A (PSA) is another important diet-independent immunomodulatory molecule. Administration of PSA to GF mice induces T-cell expansion and corrects T_H1-T_H2 imbalance in the spleen[72]. In the gut immune system, PSA increases IL-10 production by $CD4^+$ T cells and reciprocally suppresses the IL-17A response through binding to TLR2 and eventually protects against experimental colitis induced by colonization with *Helicobacter*